HAMIDIYE MEDICAL JOURNAL

The Official Journal of University of Health Sciences Türkiye, Hamidiye Faculty of Medicine

E-ISSN: 2718-0956

August - 2022
Volume - 3
Issue - 2

ORIGINAL ARTICLES

galenos Publishing House

www.hamidiyemedj.com

hamidiye med j

HAMIDIYE MEDICAL JOURNAL

2022
Volume 3

Editorial Board

Owner

Erdoğan ÇETİNKAYA
Dean of Hamidiye Faculty of Medicine, İstanbul, Türkiye
E-mail: erdogan.cetinkaya@sbu.edu.tr
ORCID: orcid.org/0000-0002-0891-0020

Editor in Chief

Zafer KARTALOĞLU
University of Health Sciences Türkiye, Hamidiye Faculty of Medicine; İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital, İstanbul, Türkiye
E-mail: zkartaloglu@gmail.com
ORCID: orcid.org/0000-0002-2954-6168

Responsible Manager

Zafer KARTALOĞLU
University of Health Sciences Türkiye, Hamidiye Faculty of Medicine; İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital, İstanbul, Türkiye
E-mail: zkartaloglu@gmail.com
ORCID: orcid.org/0000-0002-2954-6168

Associate Editor

Ebru KALE
Vice Dean of Hamidiye Faculty of Medicine, İstanbul, Türkiye
E-mail: ebru.kale@sbu.edu.tr
ORCID: orcid.org/0000-0003-1218-4962

Güven BEKTEMUR
Vice Dean of Hamidiye Faculty of Medicine, İstanbul, Türkiye
E-mail: *guven.bektemur@sbu.edu.tr*
ORCID: orcid.org/0000-0001-5899-566X

Fatih ÖZÇELİK
University of Health Sciences Türkiye, İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital, İ stanbul, Türkiye
E-mail: 68ozcelik@gmail.com - fatih.ozcelik@sbu.edu.tr
ORCID: orcid.org/0000-0003-2439-3964

Muhammed KESKİN
Bahçeşehir University, Faculty of Medicine, İstanbul, Türkiye
E-mail: drmuhammedkeskin@gmail.com
ORCID: orcid.org/0000-0002-4938-0097

Serhat PUSAT
University of Health Sciences Türkiye, Hamidiye Faculty of Medicine; İstanbul Sultan 2. Abdulhamid Han Training and Research Hospital, İstanbul, Türkiye
E-mail: pusatserhat@yahoo.com
ORCID: orcid.org/0000-0003-2412-2320

International Reviewer Board

Anesthesiology and Reanimation
Tritan Shehu
The Parlimant of Albania, Tiran, Albania

Brain Surgery
Salman Sharif
Department of Neurosurgery, of Liaquat National Hospital & Medical College, Karachi Pakistan

Cardiology
Cemil İzgi
Cardiovascular Magnetic Resonance Unit, Royal Brompton Hospital, London-UK

Cardiovascular Surgery
Mehmet Hakan Akay
Director of Minimal Invasive Cardiac Surgery Center for Advanced Heart Failure, Memorial Hermann Hospital

Family Practice
Zaim JATIC
Sarajevo University Faculty of Medicine, Department of Family Medicine, Bosnia and Herzegovina

Emergency Medicine
Roger Dickerson
Emergency Centre New Somerset Hospital, Division of Emergency Medicine University of Cape Town South Africa

Eye diseases
Levent Akduman
Department of ophtalmology SSM Health St. Louis University Hospital. Saint Louis IL, USA

Internal Diseases, Chest Diseases, Intensive Care and Sleep Medicine
Gökhan Mutlu
University of Chicago Medicine - Section of Pulmonary and Critical Care Chicago, IL, USA

Medical Genetics
Ender Mehmet Çoşkunpınar
University of Health Sciences Türkiye, Deparment of Medical Biology

Seval Türkmen
Head of the Unit Hematoonco Genetics, National Centre of Genetics, LNS Berlin, Germany

Medical Pathology
Olca Baştürk
Memorial Sloan Kettering Cancer Center, 1275 York Avenue

Medical Pharmacology
Asif Ahmed
Aston Medical Research Institute - Translational Medicine Research Group- Aston Medical School, Aston University, Birmingham, UK.

Colin Murdoch
Systems Medicine, School of Medicine, University of Dundee, Dundee, Scotland DD1 9SY, UK.

Neurology
Eric Eggenberger
Mayo Clinic Department of Neurology, Jacksonville, FL, USA

Gulnora Rakhimbaeva
Dsc Head of Neurology Department of Tashkent Medical Academy, President of Movement Disease Socity of Uzbekistan

Neonatology
Sagynbu Abduvalieva
National Birth and Childhood Center, Head of Neonatal and Premature Babies Pathology Department Kazakhstan

Orthopedics
Cebrail Alekberov
Azerbaijan Orthopedics & Traumatology Research Institute, Azerbaijan

Pediatric Diseases
Dilorom Ahmedova
Director of the National Specialized Child Health and Diseases Research Center Uzbekistan

Pediatric Rheumatology
Hajrija Maksic
Clinical Center University of Sarajevo Department of Neonatology, Bosnia and Herzegovina

Pediatric Surgery
Barbara Ludwikowski
Auf Der Bult Kinder - Und Jugendkrankenhaus, Hannover / 2. Medizinische Hoshschule Hannover, Germany

Psychiatry
Joseph Zohar
Sheba Medical Center, Tel Aviv University, Israel

Radiology
Aytekin Oto
The University of Chicago, Department of Radiology Chicago, IL, USA

Radiology (Pediatric)
Korgün Koral
University of Texas Southwestern Medical Center and Childrens Health, Pediatric Neuroradiology, USA

Thoracic Surgery
Haluk Bükesoy
Helios University Hospital Wuppertal Germany

Statistic Editor

Kürşad Nuri Baydili
University of Health Sciences Türkiye, Hamidiye Faculty of Medicine, Department of Biostatistics, İstanbul, Türkiye
E-mail: kursatnuri.baydili@sbu.edu.tr

Publisher Contact
Address: Molla Gürani Mah. Kaçamak Sk. No: 21/1 34093 İstanbul, Türkiye
Phone: +90 (212) 621 99 25 **Fax:** +90 (212) 621 99 27 **E-mail:** info@galenos.com.tr
Web: www.galenos.com.tr **Publisher Certificate Number:** 14521
Online Publication Date: August 2022 **E-ISSN:** 2718-0956
International periodical journal published three times in a year.

hamidiye med j

2022/Volume 3

HAMIDIYE MEDICAL JOURNAL

Aims and Scope

Hamidiye Medical Journal (Hamidiye Med J) is a peer-reviewed open-access international journal that publishes conducted in all fields of medicine, interesting case reports, and clinical images, invited reviews, editorials, letters, comments and letters to the Editor including reports on publication and research ethics. The journal is the official scientific publication of the University of Health Sciences Türkiye, Hamidiye Faculty of Medicine, İstanbul, Türkiye. It is published three times a year in April, August, and December. The language of the journal is English.

The journal is based on independent and unbiased double-blinded peer-reviewed principles. Only unpublished papers that are not under review for publication elsewhere can be submitted. Hamidiye Medical Journal does not accept multiple submissions and duplicate submission even though the previous one was published in a different language. The authors are responsible for the scientific content of the material to be published. Hamidiye Medical Journal reserves the right to request any research materials on which the paper is based.

Hamidiye Medical Journal encourages and enables academicians, researchers, specialists, and primary care physicians to publish their valuable research in all branches of medicine. The primary aim of the journal is to publish original articles with high scientific and ethical quality and serve as a good example of medical publications in the Türkiye as well as in the world.

Hamidiye Medical Journal is indexed in **EBSCO Host, J-Gate** and **Ulakbim TR Index.**

English Title: Hamidiye Medical Journal

Official abbreviation: Hamidiye Med J

E-ISSN: 2718-0956

OPEN ACCESS POLICY

This journal provides immediate open access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge.

Author (s) and copyright owner (s) grant access to all users for the articles published in Hamidiye Medical Journal as free of charge. Articles may be used provided that they are cited.

Open Access Policy is based on rules of Budapest Open Access Initiative (BOAI). By "open access" to [peer-reviewed research literature], we mean its free availability on the public internet, permitting any users to read, download, copy, distribute, print, search, or link to the full texts of these articles, crawl them for indexing, pass them as data to software, or use them for any other lawful purpose, without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. The only constraint on reproduction and distribution, and the only role for copyright in this domain, should be to give authors control over the integrity of their work and the right to be properly acknowledged and cited.

Creative Commons

A Creative Commons license is a public copyright license that provides free distribution of copyrighted works or studies. Authors use the CC license to transfer the right to use, share or modify their work to third parties. This journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC-ND 4.0) (https://creativecommons.org/licenses/by-nc-nd/4.0/) which permits third parties to share and adapt the content for non-commerical purposes by giving the apropriate credit to the original work.

Open access is an approach that supports interdisciplinary development and encourages collaboration between different disciplines. Therefore, Hamidiye Medical Journal contributes to the scientific publishing literature by providing more access to its articles and a more transparent review process.

Advertisement Policy

Potential advertisers should contact the Editorial Office. Advertisement images are published only upon the Editor-in-Chief's approval.

Material Disclaimer

Statements or opinions stated in articles published in the journal do not reflect the views of the editors, editorial board and/or publisher; The editors, editorial board and publisher do not accept any responsibility or liability for such materials. All opinions published in the journal belong to the authors.

Contact
Editorial Office
Address for Correspondence

Selimiye Mah. Tıbbiye Cad. 34668 Üsküdar, İstanbul/Türkiye

Phone: +90 216 418 96 16

E-mail: tipfakultesi@sbu.edu.tr

Publisher/Issuing Body

Galenos Yayınevi Tic. Ltd. Şti.

Molla Gürani Mah. Kaçamak Sok. No: 21, 34093, Fındıkzade, İstanbul, Turkiye

Phone: +90 212 621 99 25

Fax: +90 212 621 99 27

Web page: http://www.galenos.com.tr

E-mail: info@galenos.com.tr

OPEN ACCESS

hamidiye med j

HAMIDIYE MEDICAL JOURNAL

2022
Volume 3

Contents

hamidiye med j

HAMIDIYE MEDICAL JOURNAL

2022
Volume 3

Contents

REVIEW

DOI: 10.4274/hamidiyemedj.galenos.2022.76476

Hamidiye Med J 2022;3(2):76-81

Anatomy of Femoroacetabular Impingement Syndrome

Femoroasetabuler Sıkışma Sendromu Anatomisi

Özlem Öztürk Köse[1], Burak Karip[2], Fatmanur Atik[3]

[1]*Biruni University Faculty of Medicine, Department of Anatomy, İstanbul, Türkiye*

[2]*University of Health Sciences Türkiye Hamidiye Faculty of Medicine, Department of Anatomy, İstanbul, Türkiye*

[3]*Marmara University Atatürk Faculty of Education, Department of Art and Crafts Teacher Education, İstanbul, Türkiye*

ABSTRACT

Hip joint; it is a spheroid type joint formed between the head of femur and acetabular fossa. Auxiliary structures such as ligaments and joint capsule are organized to protect the hip joint. When one or more of the structures that form or protect the hip joint are damaged, torn or worn and compressed, the patient experiences significant pain and a decrease or limitation in activities of daily living. Conditions such as walking disturbances, difficulties in sitting or standing up are common. Osteoarthritis and femoroacetabular impingement syndrome (FAIS) the most common joint disorders. It is known that this syndrome is one of the first stage findings of osteoarthritis. The type of treatment is determined by looking at the radiological images of the patients, the findings in the examination and the condition of the surrounding structures. In cases where surgical intervention is required, a physical therapy program is organized according to the need after the invasive procedure as a result of the procedure. If the patient's clinical condition is not suitable for surgery and there is an indication for drug therapy, this method may also be preferred. FAIS has 3 different subtypes as cam, pincer and mixed. In the personalized medical treatments to be performed according to the different types mentioned, it is aimed that the patients continue their lives comfortably. Careful and correct use of information on the anatomy of the hip joint also positively affects the lives of patients with such disorders. Our study aims to provide a detailed guide to the topographic anatomy of the relevant region for those who conduct research in this field, especially clinicians.

Keywords: Art. coxae, femoroacetabular impingement syndrome, osteoarthritis, femur, acetabulum

ÖZ

Articulatio coxae; caput femoris ve fossa acetabuli arasında kurulan sferoid tipte bir eklemdir. Ligamentler ve eklem kapsülü gibi ekleme yardımcı oluşumlar, art. coxae'yı korumak üzere organize olmaktadır. Kalça eklemini oluşturan ya da koruyan yapılardan biri veya birkaçı hasarlandığı, koptuğu ya da aşınıp sıkıştığı zaman hastada önemli derecede ağrı ve günlük yaşam aktivitelerinde azalma veya kısıtlanma meydana gelir. Yürüyüş bozuklukları, oturma ya da yerinden kalkmada yaşanan zorluklar gibi durumlar sıkça görülür. Eklem rahatsızlıklarının en başında osteoartrit ve femoroasetabuler sıkışma sendromu (FASS) gelir ki; bu sendromun osteoartritin ilk evre bulgularından biri olduğu bilinmektedir. Hastaların radyolojik görüntülerine, muayenelerindeki bulgulara ve civar yapıların durumuna bakılarak tedavi şekli belirlenir. Cerrahi girişime ihtiyaç duyulan durumlarda, prosedür gereği invaziv girişim sonrasına yönelik, ihtiyaca göre fizik tedavi programı düzenlenmektedir. Eğer hastanın klinik durumu ameliyata elverişli değil ve ilaçlı tedavi yöntemi için endikasyon varsa, bu yöntem de tercih edilebilir. FASS'nin cam, pincer ve miks olarak 3 farklı alt tipi bulunmaktadır. Bahsedilen farklı türlere göre yapılacak olan kişiye özel medikal tedavilerde hastaların hayatlarını konforlu bir şekilde sürdürmeleri amaçlanır. Kalça ekleminin anatomisine ilişkin bilgilerin dikkatli ve doğru kullanılması, yine bu tür rahatsızlıklara sahip hastaların yaşamlarını pozitif yönde etkilemektedir. Çalışmamız, bu alanda araştırmalarını yürütenlere, özellikle de klinisyenlere ilgili bölgenin topografik anatomisine dair detaylı bir rehber olmayı amaçlamaktadır.

Anahtar Kelimeler: Art. coxae, femoroasetabuler sıkışma sendromu, osteoartrit, femur, acetabulum

Address for Correspondence: Burak Karip, University of Health Sciences Türkiye Hamidiye Faculty of Medicine, Department of Anatomy, İstanbul, Türkiye
Phone: +90 538 860 94 10 E-mail: krpbrk@gmail.com **ORCID ID:** orcid.org/0000-0002-6757-4960

Received: 16.03.2022 **Accepted:** 09.05.2022

©Copyright 2022 by University of Health Sciences Türkiye Hamidiye Faculty of Medicine. / Hamidiye Medical Journal published by Galenos Yayınevi.

Introduction

Acetabulum

The acetabulum is a cavity formed by the joint participation of 3 parts (ilium, ischium and pubis) of the hip bone that is the largest bone in the human body. When we look at these three parts in terms of participation rates in the acetabulum; ilium; superior 2/5, pubis; superior-anterior 1/5, ischium; it forms the posterior inferior 2/5 and the floor of the cupping. The peculiarity of this region is that it is the region where the hip bone articulates with the femoral head (Figure 1). The lunate surface (half moon), located on the inner surface of the acetabular fossa, is where the articular surface of the femoral head coincides. As a result of the mentioned half-moon shape, there is an acetabular notch inferior to the acetabulum and transverse acetabular ligament, which creates a hole by joining this notch surface to the lunate surface. The acetabular branch of the obturator artery and medial circumflex femoral artery pass through the mentioned hole (1). Except for the lunate surface, the region corresponding to the base of the acetabulum is not included in the insertion and the cartilage tissue is located here. It has been reported that various ossicles can be found in the acetabular fossa. Usually, this happens within the context of a varied and unexpected ossification process. The appearance of these ossicles mimics osteochondritis dissecans, post-traumatic bone fractures or degenerative disorders in the bones (2). Depending on the outer edge of the acetabulum, the acetabular labrum, which can be observed as 66% triangular, 11% round and 9% flat, is thought to contribute to joint stabilization by deepening the acetabulum with its fibrocartilaginous structure. Having these functions is a result of the fact that it increases the contact surface area for the hip joint and causes the most ideal distribution of the synovial fluid to the joint surfaces, as it provides a leak-proof structure. With a general evaluation, this situation facilitates the nutrition of the articular cartilage and helps to reduce the intra-articular friction. Additionally, the acetabular labrum may be hypoplastic or absent in 10% of the cases. From a clinical point of view, sublabral sulcus was observed in 25% of cases with suspected acetabular labrum tear. It is reported that 44% of these observed sulci are in the anterior-upper, 48% posterior-lower, 4% anterior-lower and 4% posterior-upper regions. The presence of the perilabral dead end, another potential space between the joint capsule and the acetabular labrum, can also mimic a labrum tear. A complex arterial relationship around the hip joint consists of some articular branches that come from the obturator, medial circumflex, femoral, superior gluteal, and inferior gluteal arteries (1,2).

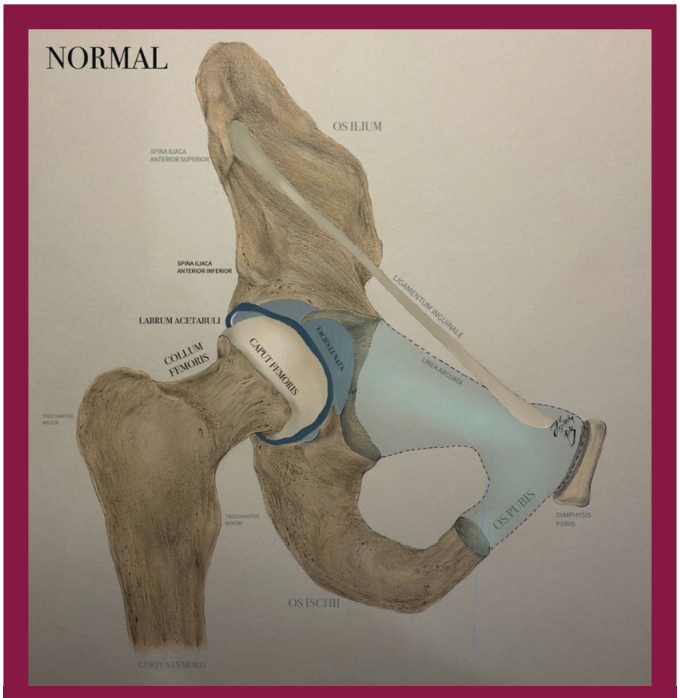

Figure 1. Normal view of the hip joint. Joint structures are femoral head and acetabulum

Femur

The other part of the hip joint other than the acetabulum is the femur, the strongest and longest bone of the human body. The part of the femur that articulates with the hip bone proximally is the femoral head. The proximal part of the femur, including the femoral head, undergoes complete fusion between the ages of 15-20. Considering the projection of this section, when we connect the superior of the greater trochanter and the pubic tubercle with a horizontal line, it can be marked approximately 2-4 cm superior to the midpoint of this line. The entire surface of the femoral head, which has a mutually curved structure with the acetabulum, is covered with cartilage tissue, except for the ligament of head of femur, which connects to the fovea for ligament. The thickness of this cartilage tissue becomes the most voluminous on the anterior-outer surface. The cartilage tissue thickness in the acetabulum, on the other hand, is at its maximum on the anterior-upper surface. As can be seen, these two voluminous surfaces are considered as the main load bearing areas in the hip joint.

Femoroacetabular Impingement Syndrome (FAIS)

The fibrous joint capsule, which surrounds the femoral head and collum femoris laterally, extends to the intertrochanteric line anteriorly, femoral neck base superiorly, intertrochanteric crest posteriorly, and attaches

at a level that coincides with the greater trochanter inferiorly, attracts attention with its dense and strong structure. attracts. In addition to the mentioned density, it is noticeably thicker in the anterior-upper region, where it is subjected to high loads. In the opposite direction, it has a thinner and looser attachment in the posterior-lower region. This relationship in the hip region can cause 2 different syndromes. The first of these; it is FAIS that causes stress to the joint due to pathology at the junction of the acetabulum and the femoral head and femoral neck (3). Developmental anomalies and variants of the femur and acetabulum can also cause FAIS. This can lead to diseases such as osteoarthritis or degenerations when the acetabular labrum and joint cartilages (2,4,5). The other pathology is; ischiofemoral impingement syndrome. This is a condition characterized by the compression of the soft tissue between the ischium and the area where the lesser trochanter is located, characterized by severe pain. Among the common causes of the two pathologies; it can be said that they constantly rub against each other during movement and that their mutual anatomical structures cannot fully adapt to each other (3). This condition, which is frequently seen in young adults, can lead to degenerations and diseases such as osteoarthritis when it is not detected and treated in the early stages, when the acetabular labrum and joint cartilage are in the advanced stages. Arthroscopy and magnetic resonance imaging are generally used in this pathology as radiological imaging. There are three subgroups of FAIS: Cam, pincer and mixed (combined). Cam type; it is the most common species and occurs when the femoral head separates from the acetabulum (Figure 2). Usually, the ligament of head of femur is ruptured and a smooth movement within the joint is not possible. An accessory bone structure in the form of a bridge is formed around the femoral head, and this structure can be damaged by grinding the articular cartilage, especially during walking. In pincer-type impingement, the femoral head is over articulated to the acetabular fossa (Figure 3). Therefore, due to this excessive attachment, it can be seen in the collum femoris and the acetabulum, and gait disturbances and pain occur. Problems arise to the flexion movement or abduction movement of the hip joint. An image similar to the clinical picture may occur in coxa vara. In combined types; both cam-type impingement and pincer-type impingement occur simultaneously on the ipsilateral joint (Figure 4). The accessory bone structure formed by arthroscopic surgery can be removed from the environment, the soft tissue deformity can be eliminated and the pain phenomenon in the patient can be eliminated (6).

Result

Hip joint is a synovial type joint that is basically formed between the femoral head and the acetabulum. Its structure supported by ligaments allows wide range of motion. In addition to this feature, hip jointis also one of the most

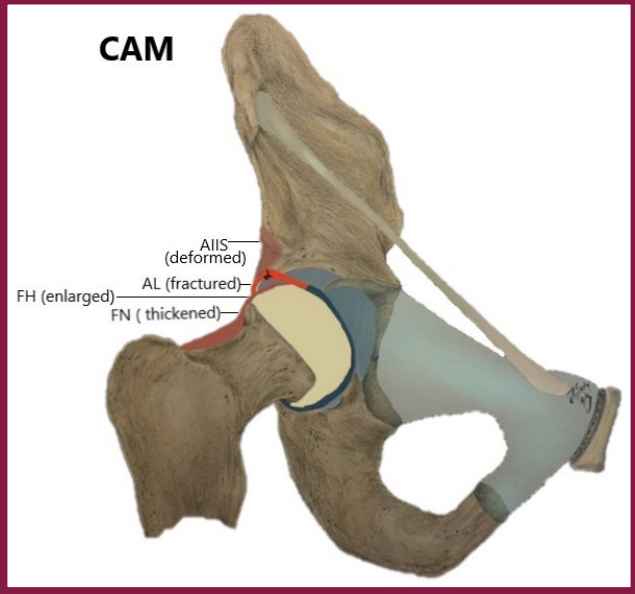

Figure 2. Cam-type FAIS. The femoral head is not perfectly round. It cannot perform rotation in the acetabulum

AIIS: Anterior inferior iliac spine, AL: Acetabular labrum, FH: Femoral head, FN: Femoral neck

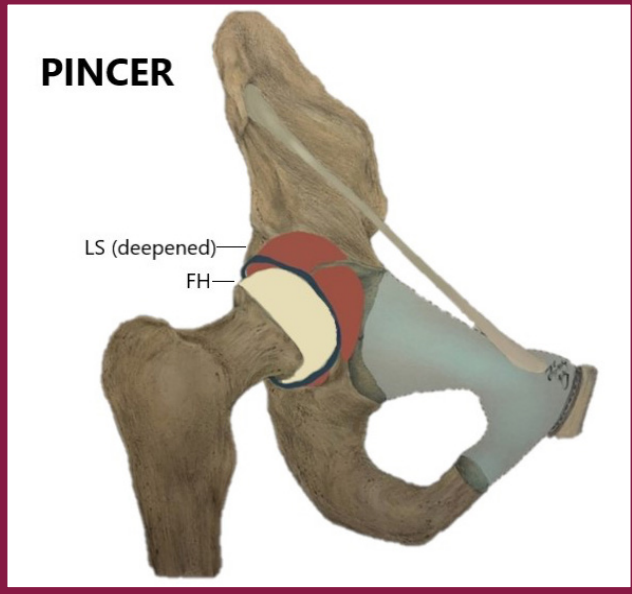

Figure 3. Pincer type FAIS. The acetabulum overly grasps the femoral head

LS: Lunate surface, FH: Femoral head, FAIS: Femoroacetabular impingement syndrome

common complaints in terms of pain due to its pathology in the body. Pain may develop due to a problem that sometimes occurs in the intracapsular region of the joint and sometimes in the extracapsular region of the joint. In any situation that causes injury or rupture of the sciatic nerve, obturator nerve and femoral nerve around the joint, the joint may be affected and the pain is felt most often in the femoral region. In addition, it is possible to feel pain in the anterior-posterior thigh, knee joint, and leg anterior-posterior regions. In addition to the damage of the mentioned nerve structures, the cause of the pain is; there may also be osteoarthritis, iliopsoas muscle compression, damage to the proximal structures of the femur, or hip compression. In addition to the pain that can be diagnosed by palpation in the physician examination, radiographic imaging methods are also used when necessary. In hip osteoaritis, which is one of the hip joint problem, the pain may start from the lumbosacral part of the spinal cord and continue to the lower part of the knee. In a study conducted with pain mapping method on 60 patients, pain distribution due to osteoarthritis in the lower extremities was examined. It has been determined that the most common pain in osteoarthritis is felt around the groin and hip; groin pain has the highest rate with 84.3%. Hip pain with osteoarthritis, which spreads due to the branching of the saphenous nerve during its downward course, is characterized by its manifestation in the lower region of the knee (7).

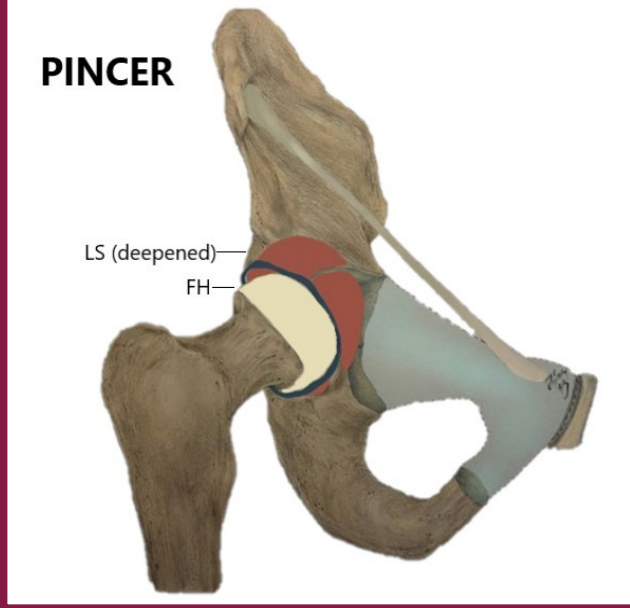

Figure 4. Mix type FAIS. Cam-type and pincer-type are seen together

LS: Lunate surface, FAIS: Femoroacetabular impingement syndrome

Osteoarthritis that can be seen in the gluteal area; it can cause atrophy of the psoas major muscle, one of the strong and important flexor muscles of this region. In such cases, pain occurs in the lower extremity and weakness in hip joint flexion may occur. It has been observed that the pain felt in the early postoperative period is reduced by arthroscopic osteochondroplasty procedures with or without acetabular labrum repair. It is noticed that the improvement followed after the pain is seen on the basis of function starting from the 6th month (8). On the other hand, (hip impingement) (FAIS), which is one of the most common cases in the hip joint, is a condition that occurs frequently due to bone deformity or some other reasons and can be seen in the first step of osteoarthritis. The prevalence of pincer and glass subgroups in asymptomatic FAIS is 67% and 37%, respectively. However, despite the relatively high prevalence of radiographic findings, fewer patients develop symptomatic FAIS syndrome or osteoarthritis than asymptomatic patients (9).

During physiological hip movement, unexpected abnormal situations may occur because the power is not shared equally in the cartilage structure and acetabular labrum (10,11). FAIS syndrome is observed in 3 different types; cam, pincer and mix. First, in the cam type of the syndrome; there is an extra bone formation on the femoral neck, but in the pincer type, various morphological reasons can be mentioned. The person may feel pain in the hip joint, back, thigh and groin areas while sitting or after exercise (12). When FAIS is evaluated on the basis of gender, it is stated that it can manifest itself as a more painful process in women than in men (13). Another study in which asymptomatic individuals were compared with symptomatic individuals; it has been determined that weakness can be observed especially in muscle strength together with the hip joint problem. However, walking problems can still occur (14). In patients with this syndrome, strengthening the damaged muscles and applying additional treatment procedures specifically applied to other muscles is thought to be an approach that has positive effects in the surgical and conservative treatment of FAIS (15). Surgical applications can lead to negative situations in which the bleeding is high volume and the surgeons viewing angles are narrowed from time to time due to the richness of the neurovascular structures in the region. Tranexamic acid, which is used in many of the practical clinical applications, has been shown to effectively reduce blood loss that occurs in arthroscopic operations and restricts surgical vision when used preoperatively (16). While planning these operations, body mass index (BMI) should be considered. According to a study, it was observed that low BMI and advanced age may be the precursors of increased pain in the follow-up

after arthroscopy treatment (17). As it can be seen, although the results of surgical applications such as arthroscopy for the treatment of FAIS in terms of quality of life and functional recovery have been studied extensively, there is a lack of sufficient information about the complications related to surgery due to surgery. In a study covering 36,761 arthroscopy operations, including surgical interventions related to FAIS, the overall complication rate was reported as 3.3%. In addition to this rate, the major complication rate was observed as 0.2% (18). When looking at the general patient group; in addition to FAIS, antalgic gait (the patient's quick movements to throw the load from the painful area to the other extremity as soon as possible) and trendelenburg syndrome (pathological gait usually caused by unilateral weakness of the m. gluteus medius) can be seen. The reason for this may be that the abductor muscles and n. gluteus inferior are affected on the problematic side. In the tests performed for the diagnosis of FAIS, the responses given to the internal rotation, external rotation, flexion and extension movements of the patient's hip joint are evaluated. However, the patient's feeling of pain in the inguinal region when the hip joint is in 90° flexion, and difficulty in adduction and internal rotation movements suggest positive anterior impingement. However, the validity of this test is not certain in impingements in the anterior-upper part of the acetabular labrum. Other methods are used for diagnosis. Ligament damage and joint instability have also been observed in the connection thought to exist between another case type, athletic pubalgia (football player's groin) disease and FAIS. In a study on cadaver pelvises, it was observed that dynamic acetabular impingement syndrome increased rotational motion on the symphysis pubis (19).

In the informative study published by the American Physical Therapy Association on the subject; it has been stated that manual therapy, stretching and core exercises can also be applied in people with FAIS. In addition, anti-inflammatory drugs can be used to prevent inflammation that may be caused by the syndrome, which can greatly reduce pain. The most frequently recommended drugs are ibuprofen, diclofenac and meloxicam active ingredients. In addition, hyaluronic acid, which is frequently used in intra-articular injections, is one of the applications that offers the patient a comfortable daily life and reduces pain in varying periods. In addition, corticosteroids, which are also administered with local anesthesia, are among the medical treatments whose duration of action varies for 9-10 days. The need for surgical intervention in patients with FAIS is usually determined by the following criteria: Hip pain and accompanying limitation of motion, positive evidence of impingement, and an alpha angle greater than 50°. In the surgical intervention, the compressed area is rasped or part of it is removed. Femoral neck osteoplasty is performed in the cam compression type. Debridements are used to repair tears in the ligaments around the hip joint (20). Although some patients have the same bone structure in the right and left hip joints in clinical terms; they may complain of pain that starts in only one side (21). Recovery rates of patients after physical therapy procedures are much lower in patients who have undergone arthroscopic surgery and then regained their health (22). So that; according to another study, after 14 months of follow-up of 1.981 patients who underwent total hip arthroplasty, it was determined that 95% of these patients returned to their daily activities and sports without any problems (23). Intraarticular pathology; since it is a condition that requires surgery, patient follow-up is an important factor.

Conclusion

As a result, since FAIS, which is characterized as one of the first steps of osteoarthritis, has been observed to have a different course between the sexes, and since it restricts mobility and greatly hinders daily life activities, the most appropriate treatment step for the patient should be preferred and applied as soon as possible. In this context, it is anticipated that our study will serve as a guide for new studies planned to be carried out with the coordination of clinical and basic sciences.

Ethics

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ö.Ö.K., B.K., F.A., Concept: Ö.Ö.K., B.K., F.A., Design: Ö.Ö.K., B.K., F.A., Data Collection or Processing: Ö.Ö.K., B.K., F.A., Analysis or Interpretation: Ö.Ö.K., B.K., F.A., Literature Search: Ö.Ö.K., B.K., F.A., Writing: Ö.Ö.K., B.K., F.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Ozan H. Premium Ozan Anatomi. 3rd ed. Nobel Tıp Kitabevleri; 2014. [Crossref]
2. Standring S. Gray's Anatomy- The Anatomical Basis of Clinical Practice. 41th ed. Standring S, editor: Elsevier; 2015. [Crossref]
3. Ahn YS, Kim S, Kim WJ, Lim JH, Jung ST. Characteristics of hip impingement syndrome in patients with multiple hereditary exostoses. BMC Musculoskelet Disord. 2021;22:153. [Crossref]
4. Cesmebasi A, Loukas M. Pelvic Bones. Bergman's Comprehensive Encyclopedia of Human Anatomic Variation; 2016;82-88. [Crossref]

5. Ward R. Hip Joint. Bergman's Comprehensive Encyclopedia of Human Anatomic Variation; 2016:176-180. [Crossref]
6. Larson CM, LaPrade RF, Floyd ER, McGaver RS, Bedi A. Acetabular Rim Disorders/Pincer-type Femoroacetabular Impingement and Hip Arthroscopy. Sports Med Arthrosc. 2021;29:35-43. [Crossref]
7. Khan AM, McLoughlin E, Giannakas K, Hutchinson C, Andrew JG. Hip osteoarthritis: where is the pain? Ann R Coll Surg Engl. 2004;86:119-121. [Crossref]
8. Almasri M, Simunovic N, Heels-Ansdell D, Ayeni OR; Fırst Investigators. Femoroacetabular impingement surgery leads to early pain relief but minimal functional gains past 6 months: experience from the FIRST trial. Knee Surg Sports Traumatol Arthrosc. 2021;29:1362-1369. [Crossref]
9. McCrum CL. Editorial Commentary: Lumbosacral Anatomy and Mechanics Influence Femoroacetabular Impingement Syndrome and Surgical Outcomes: The Hip Bone Is Connected to the Back Bone. Arthroscopy. 2021;37:156-158. [Crossref]
10. Amanatullah DF, Antkowiak T, Pillay K, Patel J, Refaat M, Toupadakis CA, et al. Femoroacetabular impingement: current concepts in diagnosis and treatment. Orthopedics. 2015;38:185-199. [Crossref]
11. Bredella MA, Ulbrich EJ, Stoller DW, Anderson SE. Femoroacetabular impingement. Magn Reson Imaging Clin N Am. 2013;21:45-64. [Crossref]
12. Zhang C, Li L, Forster BB, Kopec JA, Ratzlaff C, Halai L, et al. Femoroacetabular impingement and osteoarthritis of the hip. Can Fam Physician. 2015;61:1055-1060. [Crossref]
13. Lewis CL, Khuu A, Loverro KL. Gait Alterations in Femoroacetabular Impingement Syndrome Differ by Sex. J Orthop Sports Phys Ther. 2018;48:649-658. [Crossref]
14. Samaan MA, Zhang AL, Popovic T, Pedoia V, Majumdar S, Souza RB. Hip joint muscle forces during gait in patients with femoroacetabular impingement syndrome are associated with patient reported outcomes and cartilage composition. J Biomech. 2019;84:138-146. [Crossref]
15. Frasson VB, Vaz MA, Morales AB, Torresan A, Telöken MA, Gusmão PDF, et al. Hip muscle weakness and reduced joint range of motion in patients with femoroacetabular impingement syndrome: a case-control study. Braz J Phys Ther. 2020;24:39-45. [Crossref]
16. Li N, Qin LW, Jiang HJ. [Clinical effects of tranexamic acid in arthroscope for femoroacetabular impingement]. Zhongguo gu shang. 2021;34:121-125. [Crossref]
17. Kay J, Simunovic N, Heels-Ansdell D, Bhandari M, Ayeni OR; Fırst Investigators. Lower body mass index and age are predictive of improved pain and health utility scores following arthroscopic management of femoroacetabular impingement. Knee Surg Sports Traumatol Arthrosc. 2021;29:1461-1473. [Crossref]
18. Ohlin A, Simunovic N, Duong A, Ayeni OR; Fırst Investigators. Low rate of adverse events in a randomized controlled trial addressing the surgical treatment of femoroacetabular impingement (FAI) syndrome. Knee Surg Sports Traumatol Arthrosc. 2021;29:2015-2020. [Crossref]
19. Birmingham PM, Kelly BT, Jacobs R, McGrady L, Wang M. The effect of dynamic femoroacetabular impingement on pubic symphysis motion: a cadaveric study. Am J Sports Med. 2012;40:1113-1118.
20. Menge TJ, Truex NW. Femoroacetabular impingement: a common cause of hip pain. Phys Sportsmed. 2018;46:139-144. [Crossref]
21. Swenson K, Erickson J, Peters C, Aoki S. Hip pain in young adults: Diagnosing femoroacetabular impingement. JAAPA. 2015;28:39-45. [Crossref]
22. Gao I, Safran MR. Nonsurgical Versus Surgical Management of Femoroacetabular Impingement: What Does the Current Best Evidence Tell Us. J Am Acad Orthop Surg. 2021;29:e471-e478. [Crossref]
23. Minkara AA, Westermann RW, Rosneck J, Lynch TS. Systematic Review and Meta-analysis of Outcomes After Hip Arthroscopy in Femoroacetabular Impingement. Ame J Sports Med. 2019;47:488-500. [Crossref]

REVIEW

DOI: 10.4274/hamidiyemedj.galenos.2022.33043

Hamidiye Med J 2022;3(2):82-88

Resveratrol and Its Anticancer Effects

Resveratrol ve Antikanser Etkileri

Beyza Nur Özkan[1], Eray Metin Güler[1,2]

[1]*University of Health Sciences Türkiye Hamidiye Faculty of Medicine, Derpartment of Medical Biochemistry, İstanbul, Türkiye*

[2]*University of Health Sciences Türkiye Haydarpaşa Numune Health Application and Research Center, Clinic of Medical Biochemistry, İstanbul, Türkiye*

ABSTRACT

Cancer is a worldwide public health problem and is the second leading cause of death in the world. Resveratrol, which is an antioxidant molecule belonging to the polyphenol family, is usually extracted from a large number of natural plants. Recently, many studies have been conducted on resveratrol and its effect on cancer. In this review, the effects of resveratrol are emphasized on chemopreventive, therapeutic, and anticancer. More than 70 related scientific articles from various databases (e.g.; Science Direct, MDPI, PubMed, and Google Scholar) were evaluated for this review using the keywords anticancer, antioxidant, apoptosis, cancer, resveratrol, tumorigenesis. It has been revealed that resveratrol is associated with many biochemical pathways that are effective in the formation, development, and spread of cancer.

Keywords: Anticancer, antioxidant, apoptosis, cancer, resveratrol, tumorigenesis

ÖZ

Kanser dünya çapında bir halk sağlığı sorunudur ve dünyada ölüm nedenleri arasında ikinci sırada yer almaktadır. Polifenol ailesine ait antioksidan bir molekülü olan resveratrol, genellikle çok sayıda doğal bitkiden ekstrakte edilir. Son zamanlarda resveratrol ve kanser ile ilgili birçok çalışma yapılmıştır. Bu derlemede resveratrolün kemopreventif, terapötik ve antikanser etkileri üzerinde durulmuştur. Bu derleme çalışması için antikanser, antioksidan, apoptoz, kanser, resveratrol, tümörogenez anahtar kelimeleri kullanılarak çeşitli veri tabanlarından (örneğin; Science Direct, MDPI, PubMed ve Google Scholar) 70'ten fazla ilgili bilimsel makale değerlendirilmiştir. Resveratrolün kanser oluşumunda, gelişiminde ve yayılmasında etkili olan birçok biyokimyasal yolaklarla ilişkili olduğu ortaya koyulmuştur.

Anahtar Kelimeler: Antikanser, antioksidan, apoptoz, kanser, resveratrol, tümörogenez

Introduction

Resveratrol (3, 4', 5-trihydroxystilbene; RSV) is a stilbene phytoalexin which is a kind of natural phenol and is composed part of the defense system in plants (1). It was first isolated from the roots of *veratrum grandiflorum* by Takaoka in 1939. RSV is a polyphenolic compound with different mechanisms of action found in grapes, wine, peanuts, and blueberries (2). There are two geometric isomers of RSV as cis- (Z) and trans- (E) in Figure 1 (3). The trans isoform is biologically active. The trans form can be found industrially in cosmetic ingredients or used as a food supplement, which is obtained from yeast extracts recombinantly.

Additionally, its isomerizes to the cis form by exposing to ultraviolet radiation, light, or heat (4). Recent studies have been shown that RSV has many effects, such as antioxidative, antiinflammatory, cardioprotective, antidiabetic, anticancer, chemopreventive, and neuroprotective effects (5). It achieves all these effects by targeting tumor angiogenesis, apoptosis regulators, cell survival, metastasis and intracellular

Address for Correspondence: Eray Metin Güler, University of Health Sciences Türkiye Hamidiye Faculty of Medicine, Derpartment of Medical Biochemistry; Haydarpaşa Numune Health Application and Research Center, İstanbul, Türkiye
Phone: +90 555 377 84 76 E-mail: eraymetinguler@gmail.com **ORCID ID:** orcid.org/0000-0003-4351-1719
Received: 19.03.2022 **Accepted:** 20.06.2022

©Copyright 2022 by University of Health Sciences Türkiye Hamidiye Faculty of Medicine. / Hamidiye Medical Journal published by Galenos Yayınevi.

signaling factors, including key components such as proinflammatory mediators, a different set of transcription factors and many signaling pathway regulators (6).

Cancer is a disease characterized by abnormal, uncontrolled cell growth that has the potential to spread to other parts of the body (7). Its the second most common cause of death in the world after cardiovascular diseases. There are 19.3 million new cancer cases and approximately 10.0 million cancer-related deaths worldwide in 2020. In both sexes, lung cancer accounts for 11.6% of all cases and is the most frequently diagnosed cancer. Considering their incidences, female breast cancer (11.6%), prostate cancer (7.1%), and colorectal cancer (6.1%) are followed up lung cancer, respectively (8). The treatments recently applied to cancer patients in the clinic include immunotherapy, chemotherapy, radiotherapy, and surgical operation (9). However, all these treatment strategies can damage the cancer patients and the immune system of the person (10). Nowadays, since plants and fruits are naturally rich in beneficial components for the body, new drugs are being researched for new drug formations, and it is thought that they may be a new treatment option for many diseases that cannot be cured (11). In recent years, the effects of RSV as a functional nutritional component, which has beneficial biological effects on health and cancer, have attracted attention (12).

RSV Content of Foods

Primary dietary sources of RSV are grapes, peanuts, strawberries, and legumes (13). The major source is grapes because the compound is also found in wine which is one of the grape's end products. However, its highest levels are naturally found in the roots of Japanese Knotweed (*Polygonum cuspidatum*), which is used in traditional Asian herbal medicine (14). Today, other sources can also be identified. The RSV contents of foods show the studies carried out in Table 1 (15,16).

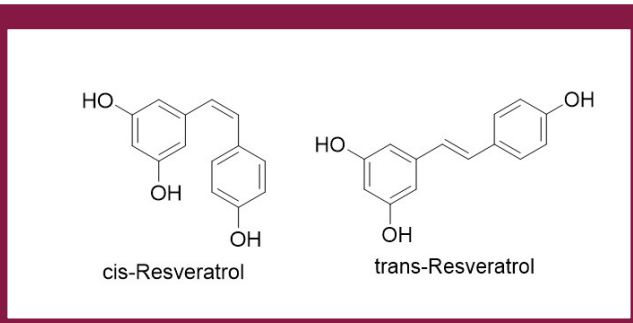

Figure 1. Cis- and trans-configuration of resveratrol

Biosynthesis and Bioavailability of RSV

There are two main pathways for RSV production, one of tyrosine (Tyr) and the other one is phenylalanine (PA) intermediates. Cinnamic acid is produced from PA by PA ammonia-lyase. The cinnamic acid is then hydroxylated with cinnamic acid 4-hydroxylase (C4H) to p-coumaric acid. Finally, cinnamic acid can be converted to RSV via 4-coumaryl-CoA lyase-1 (4CL1) and stilbene synthase (STS) (17). In the Tyr pathway, p-coumaric acid is formed by Tyr ammonium lyase from Tyr. Then it is condensed by the 3-malonyl-coA unit, and RSV is synthesized via 4CL1 and STS, such as the PA pathway in Figure 2 (18,19).

RSV is extensively metabolized by enzymes localized in the gut. Therefore, it shows a low oral bioavailability as a result of presystemic elimination. In the last decade, various methodological approaches (encapsulation, lipid nanocarriers, emulsions, etc.) have been developed to improve the low bioavailability of RSV (20).

Metabolism of RSV

The cis and trans are forms found in our daily diet, and 3-O-beta-D-glycoside is the glycosylated form of RSV. Although a small amount of RSV is absorbed by the ileum, most are absorbed by the jejunum. RSV enters the bloodstream after absorption by transmembrane transporters such as integrins or passive diffusion in the intestine and can exist in three different forms: Glucuronide, sulfate, or free form (21). It is modified by glucuronidation reaction in liver microsomes and reaches tissues. It is

Table 1. Resveratrol contents of foods

Foods	Resveratrol contents
Grape	6.47 µg/g d.m.
Grape seed extract	5.89 µg/g d.m.
Grape skin extract	3.54 µg/g d.m
Grape skin	50-100 µg/g
Grape juice	0.45-2.60 mg/L
Fresh grapes	0.16-3.54 µg/g
Raisin skin	24.06 µg/g
Red wine	0.362-1.979 mg/L
White wine	0.057-0.390 mg/L
Peanut and pistachios	0.02-1.79 µg/g
Raw peanuts	0.09-0.30 µg/g
Roasted peanuts	0-0.13 µg/g
Peanut butter	0.27-0.70 µg/g
Plum peel	0.1-6.2 µg/g
Tomato peel	18.4±1.6 µg/g d.m.
Black mulberry extract	50.61 µg/g d.m.
Lingonberry	5.88 µg/g d.m.
Cocoa	1.85±0.43 µg/g
Dark chocolate	0.35±0.08 µg/g
Chocolate milk	0.10±0.05 µg/g

d.m.: Dry matter

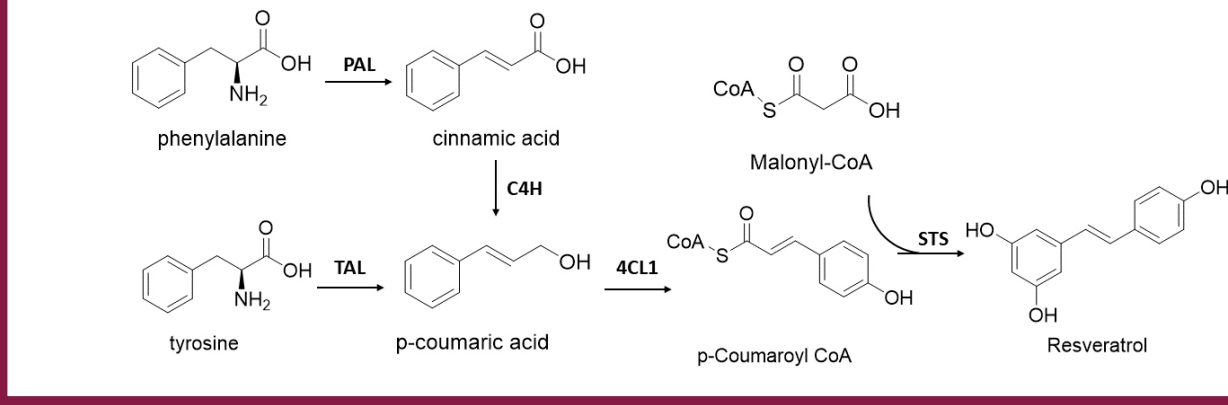

Figure 2. Biosynthesis of resveratrol
PAL: Phenylalanine ammonia-lyase, TAL: Tyrosine ammonium lyase, C4H: Cinnamic acid 4-hydroxylase, 4CL1: 4-coumaryl-CoA lyase-1, STS: Stilbene synthase

removed from the body through feces and urine in Figure 3 (22,23).

The major metabolite of RSV in humans is the sulfated metabolite resveratrol-3-O-sulfate. Other sulfated metabolites include resveratrol-4′-O-sulfate and resveratrol-3-O-4-O-disulfate. Glucuronide metabolites include resveratrol-3-O-glucuronide and resveratrol-4-O-glucuronide (24). In a study, it was observed that glucuronides were the main metabolite in plasma with low dose (5-50 mg) RSV, while monosulfates were the main metabolite in plasma with high dose (≥250 mg) RSV (25).

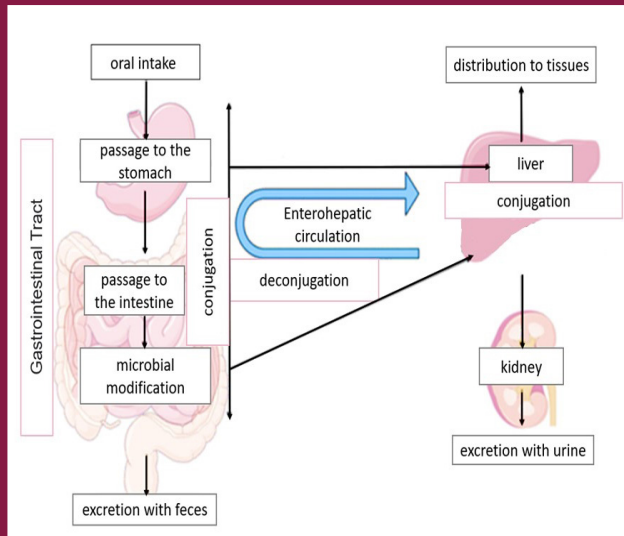

Figure 3. Metabolism and excretion of resveratrol

Pathophysiological Mechanism of RSV in Cancer

RSV has curative effects by investigating various *in vitro* and *in vivo* disease models, and this situation has ever increased the curiosity about RSV (26). At the same time, RSV targets and affects many molecules associated with human clinical conditions, such as cytokines, transcription factors, enzymes, and kinases (27). It has been observed that RSV shows anticancer activity by apoptosis, differentiation, and inhibiting cancer cell proliferation and prevents the neoplastic transformation of cells. In a study, it was shown that RSV prevents tumor angiogenesis, metastasis and also suppresses tumorigenesis phases (28). Many studies have been suggested that RSV acts apoptotic and anticancer effects on multiple cellular targets by controlling signaling pathways such as nuclear factor erythroid-2 (Nrf2), nuclear factor kappa B (NF-kB), sirtuin 1 (Sirt1), and 5'AMP-activated protein kinase (AMPK) in Figure 4 (29,30).

Modulates Apoptosis and Autophagy with Anticancer Effect

RSV plays a decisive role in cancer initiation, progression, and survival of cancer cells through modulation of apoptotic and autophagic cell death pathways (31). It activates the apoptosis mechanism by inducing the caspase enzyme system, which also regulates the expression and activity of the Atg5-Atg12-Atg16 complex required for the phagophore (32). RSV can directly activate Sirt1 expression and induce autophagy independently or dependently on the mammalian target of rapamycin (mTOR) (33). Sirt1 participates in many disease processes, including cancer and altered cellular metabolism disorders, while mainly modulating autophagy signaling. The activity of Sirt1 has an inverse relationship with mTOR (34). Moreover, Sirt1 can induce autophagy by affecting *Atg 5*, *Atg 7*, and *LC3* genes (35).

Mitogen-activated protein kinase (MAPK) is an essential regulator for the body, which is stimulated under stress and positively changes the response of cancer cells to targeted therapies and chemotherapy (36). MAPK modulates apoptosis by p38 kinase, extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNKs) pathways (37). Which is a substantial signaling pathway in tumor migration and invasion, MAPK/NF-kB is suppressed by RSV (38). Another study on rats supported that RSV reduced metastasis through this pathway (39).

Effective on Metastasis

Today, its known that epithelial-mesenchymal transition (EMT) is associated with cancer progression, invasion, and metastasis. Studies have been shown that RSV can suppress the spread and metastasis of the tumor by increasing the invasiveness of cancer cells and inhibiting the signaling pathways associated with EMT, which is claimed to be a causative agent of metastasis (40). In addition, recent *in vitro* studies have suggested that different doses of RSV can be used as a therapeutic agent by demonstrating its therapeutic properties in many different cancer types such as oral squamous cell carcinoma, colorectal, prostate, and breast cancer via EMT (41,42).

RSV Inhibits Angiogenesis

Tumor angiogenesis is modulated by angiogenic stimulants, including vascular endothelial growth factor (VEGF), an important regulatory factor in the prognosis of various cancers (43). Hypoxia-inducible factor (HIF)-1α expression increases due to a deficiency in the oxygen microenvironment, which is closely related to the development and formation of various tumor types. HIF-1α also participates in angiogenesis considerably (44). HIF-1α interact with each other to regulate VEGF expression. Due to the therapeutic potential, RSV suppresses tumor angiogenesis by inhibiting HIF-1α and VEGF protein (45). In a study, RSV 800 mg/day was given to the participants orally for 40 days. As a result, it was observed that there was a decrease in the expression of *VEGF* and *HIF-1* genes with the effect of RSV in granulosa cells (46).

Its known that the hedgehog (Hh) signaling pathway can be involved in different tumors such as pancreatic and esophageal cancers, various stages of carcinogenesis, metastatic tumors, and stimulate tumorigenesis (47). It has been determined that RSV inhibits tumor formation and metastasis by suppressing the Hh signaling pathway (48).

Modulates Inflammation-related Cancer

Cancer formation is closely related to both chronic and acute inflammation processes. The presence of inflammation can cause tumor formation and progression, neoplastic transformation, and metastasis (49). A study showed that RSV had antioxidant and antiinflammatory activities with curative effects on carcinoma by modulation of STAT3/NF-kB and Nrf2/HO-1 signaling pathways (50). STAT3 is a key

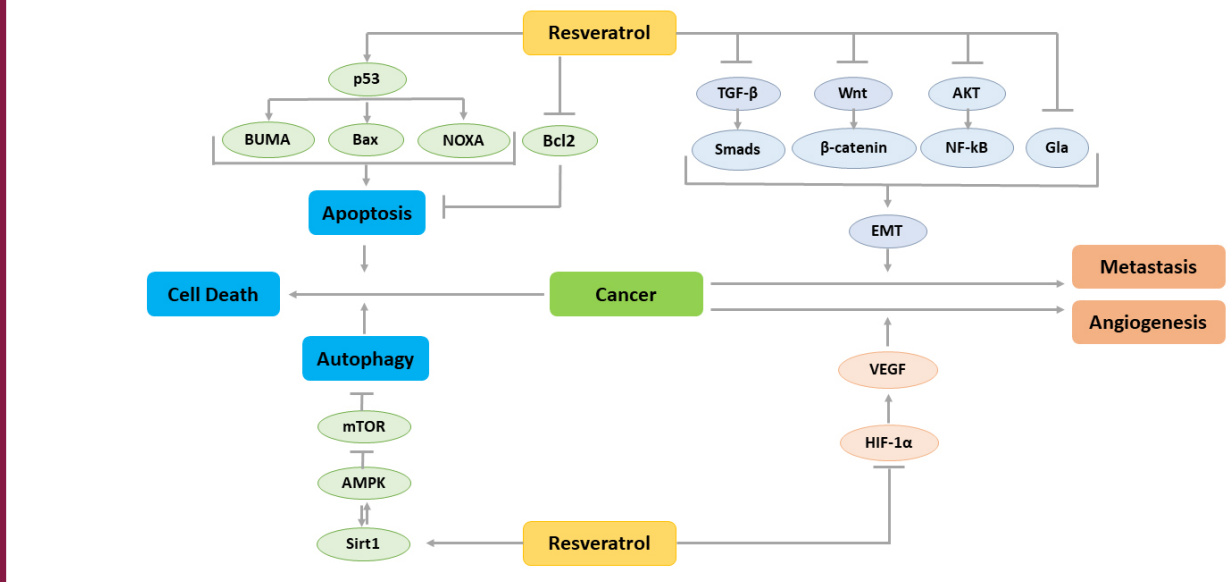

Figure 4. The relationship of resveratrol with apoptosis, autophagy, and cell death by affecting various signaling pathways

mTOR: Mammalian target of rapamycin, EMT: Epithelial-mesenchymal transition, VEGF: Vascular endothelial growth factor, AMPK: 5'AMP-activated protein kinase, Sirt1: Sirtuin 1

regulator of cell proliferation, apoptosis and is constitutively activated in cancer types. STAT3 is constitutively activated in many tumor types. Studies have shown that RSV effectively prevents cancer by inhibiting STAT3 expression (51). It also has an inhibitory effect on antiapoptotic mediators such as NF-kB, COX-2, phosphatidylinositol 3-kinase (PI3K), and mTOR (52). Activation of the Nrf2/antioxidant response element (ARE) pathway by endogenous or exogenous stimuli under normal physiological conditions has the potential to inhibit cancer and/or cancer cell survival, growth, and proliferation (53). RSV can inhibit tumorigenesis by regulating the expression of proteins, oxidase, and phase II detoxifying enzymes, which are of great importance in preventing tumorigenesis and activating the Nrf2/ARE signaling pathway (54). In addition, it can positively affect autophagy through the Nrf2/Keap1/p62 pathway and helps to regulate cellular homeostasis (55). RSV shows its anticancer effects through different mechanisms, one of which is inducing apoptosis of cancerous cells by activating apoptotic pathways via caspase proteins and programmed cell death (56). As a result of inflammation, various immune cells, such as neutrophils, macrophages, lipid cells, dendritic cells, and the release of cytokines and chemokines trigger the generation of cancer cells and cause the formation of the tumor microenvironment and tumor tissues by various pathways (57). Studies have been shown that the NLR family pyrin domain containing 3 (*NLRP3*) inflammasome gene, which is one of these pathways, plays a role in tumorigenesis. Activation of caspase-1, depending on the activation of the *NLRP3* gene causes the release of interleukin-18 (IL-18) and interleukin-1beta (IL-1β), resulting in oncological signals. Sirt1 protein is known to attenuate *NLRP3* inflammasome gene-dependent inflammation and pyroptosis through metabolic modulation. RSV downregulates the *NLRP3* gene by activating the Sirt1 protein, thereby inducing autophagy (58). In a study, it has been observed that RSV has an anti-tumor effect by suppressing the activity of NLRP3 in renal cancer cells (59).

Inhibiting Cancer Formation by Regulating Oxidative and Genotoxic Stress

Oxidative stress, which is defined as the imbalance between the increase in ROS production and the capacity of antioxidant systems to scavenge free radicals, is considered to strengthen carcinogenesis (60,61). However, RSV has a critical role as an antioxidant due to its ability to inhibit lipid peroxidation induced by the Fenton reaction, scavenging oxidants and free radicals, reducing oxidative reactions, and increasing the activity of antioxidant enzymes (62). RSV can improve the clinical outcome of certain cancers by downregulating COX-2 expression by acting on the NF- kB and activator protein-1 (AP-1) complex transcription factors with the aid of kinases such as MAPK/ERK/p38/JNK as a cancer preventative (63,64).

While antioxidants reduce oxidative stress at low doses, they have therapeutic doses that can increase the selective death of cancer cells and the effectiveness of standard treatment by increasing ROS production with a prooxidative effect at high doses (65). Studies have been shown that RSV at different concentrations offers favorable suppression of cancer generation and cancer treatment as RSV mediates cytotoxicity in cancer cells by increasing intracellular hydrogen peroxide (H_2O_2) and oxidative stress levels that will cause cell death (66). Its also known that RSV inhibits constitutive cyclooxygenase-1 but is not inducible to COX-2. Cyclooxygenases stimulate cell proliferation by producing prostaglandins from arachidonic acid, and tumor growth by angiogenesis and immunosuppression. Its thought that they can be used as therapeutic agents against various cancers by inhibiting cyclooxygenases (67).

The p53 protein has an important role in cell cycle arrest in response to genotoxic stress and inhibiting the development of carcinogenesis by inducing apoptosis (68). Studies have been affirmed that RSV activates p53, increases the expression of PUMA and BAX by activating an unknown signal pathway in addition to the p53-dependent pathway, and facilitates apoptosis by regulating the transcription of target genes involved in DNA repair (69). Similar to some chemotherapeutic drugs and radiotherapy, RSV induces DNA damage in cancer cells. Various cell death initiating enzymes are activated through signaling pathways by accumulating unrepaired DNA breaks in targeted cancer cells (70).

Conclusion

RSV is an ascendant compound that is effective in signal transduction pathways in the formation, development, and invasion stages of cancer cells. Its seen that RSV has anticancer effects with many mechanisms and signaling pathways. Account of all these properties, its thought to be a promising agent in the treatment of cancer disease. In order to benefit from its protective effects, foods containing RSV such as grapes, strawberries, blueberries, peanuts, and cocoa should be included in the diet. In addition to all these beneficial effects, its also known that RSV has side effects due to excessive intake. However, research is insufficient despite many studies on RSV and its effects on cancer. New studies are needed to explain this relationship and even to reveal the possible unknown beneficial effects of RSV.

Ethics

Peer-review: Internally peer-reviewed.

Authorship Contributions

Literature Search: B.N.Ö., E.M.G., Writing: B.N.Ö., E.M.G.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Hasan MM, Cha M, Bajpai VK, Baek KH. Production of a major stilbene phytoalexin, resveratrol in peanut (Arachis hypogaea) and peanut products: a mini review. Reviews in Environmental Science and Bio/Technology. 2013;12:209-221. [Crossref]
2. Kiran TR, Otlu O, Karabulut E, Karabulut AB. Protective Effects of Grape Molasses and Resveratrol Against DMBA Induced Oxidative Stress in Rat Ovarian Tissues. Middle Black Sea Journal of Health Science. 2019;5:151-159. [Crossref]
3. Tian B, Liu J. Resveratrol: a review of plant sources, synthesis, stability, modification and food application. J Sci Food Agric. 2020;100:1392-1404. [Crossref]
4. Repossi G, Das UN, Eynard AR. Molecular Basis of the Beneficial Actions of Resveratrol. Arch Med Res. 2020;51:105-114. [Crossref]
5. Delpino FM, Figueiredo LM, Caputo EL, Mintem GC, Gigante DP. What is the effect of resveratrol on obesity? A systematic review and meta-analysis. Clin Nutr ESPEN. 2021;41:59-67. [Crossref]
6. Hecker A, Schellnegger M, Hofmann E, Luze H, Nischwitz SP, Kamolz LP, et al. The impact of resveratrol on skin wound healing, scarring, and aging. Int Wound J. 2022;19:9-28. [Crossref]
7. Willis RE. Targeted Cancer Therapy: Vital Oncogenes and a New Molecular Genetic Paradigm for Cancer Initiation Progression and Treatment. Int J Mol Sci. 2016;17:1552. [Crossref]
8. Yu WD, Sun G, Li J, Xu J, Wang X. Mechanisms and therapeutic potentials of cancer immunotherapy in combination with radiotherapy and/or chemotherapy. Cancer Lett. 2019;452:66-70. [Crossref]
9. Graham K, Unger E. Overcoming tumor hypoxia as a barrier to radiotherapy, chemotherapy and immunotherapy in cancer treatment. Int J Nanomedicine. 2018;13:6049-6058. [Crossref]
10. Schirrmacher V. From chemotherapy to biological therapy: A review of novel concepts to reduce the side effects of systemic cancer treatment (Review). Int J Oncol. 2019;54:407-419. [Crossref]
11. Bhanot A, Sharma R, Noolvi MN. Natural sources as potential anticancer agents: A review. International Journal of Phytomedicine. 2011;3:9-26. [Crossref]
12. Xin ZH, Yang HH, Gan YH, Meng YL, Li YP, Ge LP, et al. Finding a resveratrol analogue as potential anticancer agent with apoptosis and cycle arrest. J Pharmacol Sci. 2020;143:238-241. [Crossref]
13. Shrikanta A, Kumar A, Govindaswamy V. Resveratrol content and antioxidant properties of underutilized fruits. J Food Sci Technol. 2015;52:383-390. [Crossref]
14. Stervbo U, Vang O, Bonnesen C. A review of the content of the putative chemopreventive phytoalexin resveratrol in red wine. Food Chemistry. 2007;101:449-457. [Crossref]
15. Dybkowska E, Sadowska A, Swiderski F, Rakowska R, Wysocka K. The occurrence of resveratrol in foodstuffs and its potential for supporting cancer prevention and treatment. A review. Rocz Panstw Zakl Hig. 2018;69:5-14. [Crossref]
16. Weiskirchen S, Weiskirchen R. Resveratrol: How Much Wine Do You Have to Drink to Stay Healthy? Adv Nutr. 2016;7:706-718. [Crossref]
17. Sáez-Sáez J, Wang G, Marella ER, Sudarsan S, Cernuda Pastor M, Borodina I. Engineering the oleaginous yeast Yarrowia lipolytica for high-level resveratrol production. Metab Eng. 2020;62:51-61. [Crossref]
18. He Q, Szczepańska P, Yuzbashev T, Lazar Z, Ledesma-Amaro R. De novo production of resveratrol from glycerol by engineering different metabolic pathways in Yarrowia lipolytica. Metab Eng Commun. 2020;11:e00146. [Crossref]
19. Riccio BVF, Spósito L, Carvalho GC, Ferrari PC, Chorilli M. Resveratrol isoforms and conjugates: A review from biosynthesis in plants to elimination from the human body. Arch Pharm (Weinheim). 2020;353:e2000146. [Crossref]
20. Chimento A, De Amicis F, Sirianni R, Sinicropi MS, Puoci F, Casaburi I, et al. Progress to Improve Oral Bioavailability and Beneficial Effects of Resveratrol. Int J Mol Sci. 2019;20:1381. [Crossref]
21. Gambini J, Inglés M, Olaso G, Lopez-Grueso R, Bonet-Costa V, Gimeno-Mallench L, et al. Properties of Resveratrol: In Vitro and In Vivo Studies about Metabolism, Bioavailability, and Biological Effects in Animal Models and Humans. Oxid Med Cell Longev. 2015;2015:837042. [Crossref]
22. Bird JK, Raederstorff D, Weber P, Steinert RE. Cardiovascular and Antiobesity Effects of Resveratrol Mediated through the Gut Microbiota. Adv Nutr. 2017;8:839-849. [Crossref]
23. Baltaci SB, Mogulkoc R, Baltaci AK. Resveratrol and exercise. Biomed Rep. 2016;5:525-530. [Crossref]
24. Wang P, Sang S. Metabolism and pharmacokinetics of resveratrol and pterostilbene. Biofactors. 2018;44:16-25. [Crossref]
25. Cottart CH, Nivet-Antoine V, Beaudeux JL. Review of recent data on the metabolism, biological effects, and toxicity of resveratrol in humans. Mol Nutr Food Res. 2014;58:7-21. [Crossref]
26. Shukla Y, Singh R. Resveratrol and cellular mechanisms of cancer prevention. Ann N Y Acad Sci. 2011;1215:1-8. [Crossref]
27. Lee SY, Lee SJ, Yim DG, Hur SJ. Changes in the Content and Bioavailability of Onion Quercetin and Grape Resveratrol During In Vitro Human Digestion. Foods. 2020;9:694. [Crossref]
28. Kim CW, Hwang KA, Choi KC. Anti-metastatic potential of resveratrol and its metabolites by the inhibition of epithelial-mesenchymal transition, migration, and invasion of malignant cancer cells. Phytomedicine. 2016;23:1787-1796. [Crossref]
29. Elshaer M, Chen Y, Wang XJ, Tang X. Resveratrol: An overview of its anti-cancer mechanisms. Life Sci. 2018;207:340-349. [Crossref]
30. Zhuang Y, Wu H, Wang X, He J, He S, Yin Y. Resveratrol Attenuates Oxidative Stress-Induced Intestinal Barrier Injury through PI3K/Akt-Mediated Nrf2 Signaling Pathway. Oxid Med Cell Longev. 2019;2019:7591840. [Crossref]
31. Patra S, Pradhan B, Nayak R, Behera C, Rout L, Jena M, et al. Chemotherapeutic efficacy of curcumin and resveratrol against cancer: Chemoprevention, chemoprotection, drug synergism and clinical pharmacokinetics. Semin Cancer Biol. 2021;73:310-320. [Crossref]
32. Jia B, Zheng X, Wu ML, Tian XT, Song X, Liu YN, et al. Increased Reactive Oxygen Species and Distinct Oxidative Damage in Resveratrol-suppressed Glioblastoma Cells. J Cancer. 2021;12:141-149. [Crossref]
33. Yao Q, Wu Q, Xu X, Xing Y, Liang J, Lin Q, et al. Resveratrol Ameliorates Systemic Sclerosis via Suppression of Fibrosis and Inflammation Through Activation of SIRT1/mTOR Signaling. Drug Des Devel Ther. 2020;14:5337-5348. [Crossref]
34. Maiese K. Moving to the Rhythm with Clock (Circadian) Genes, Autophagy, mTOR, and SIRT1 in Degenerative Disease and Cancer. Curr Neurovasc Res. 2017;14:299-304. [Crossref]
35. Pineda-Ramírez N, Aguilera P. Resveratrol as an inductor of autophagy: is there a unique pathway of activation? Neural Regen Res. 2021;16:101-103. [Crossref]

36. Lee S, Rauch J, Kolch W. Targeting MAPK Signaling in Cancer: Mechanisms of Drug Resistance and Sensitivity. Int J Mol Sci. 2020;21:1102. [Crossref]
37. Cenariu D, Fischer-Fodor E, Ţigu AB, Bunea A, Virág P, Perde-Schrepler M, et al. Zeaxanthin-Rich Extract from Superfood Lycium barbarum Selectively Modulates the Cellular Adhesion and MAPK Signaling in Melanoma versus Normal Skin Cells In Vitro. Molecules. 2021;26:333. [Crossref]
38. Tong W, Chen X, Song X, Chen Y, Jia R, Zou Y, et al. Resveratrol inhibits LPS-induced inflammation through suppressing the signaling cascades of TLR4-NF-κB/MAPKs/IRF3. Exp Ther Med. 2020;19:1824-1834. [Crossref]
39. Zhang X, Wang Y, Xiao C, Wei Z, Wang J, Yang Z, et al. Resveratrol inhibits LPS-induced mice mastitis through attenuating the MAPK and NF-κB signaling pathway. Microb Pathog. 2017;107:462-467. [Crossref]
40. Song Y, Chen Y, Li Y, Lyu X, Cui J, Cheng Y, et al. Resveratrol Suppresses Epithelial-Mesenchymal Transition in GBM by Regulating Smad-Dependent Signaling. BioMed Res Int. 2019;2019:1321973. [Crossref]
41. Bang TH, Park BS, Kang HM, Kim JH, Kim IR. Polydatin, a Glycoside of Resveratrol, Induces Apoptosis and Inhibits Metastasis Oral Squamous Cell Carcinoma Cells In Vitro. Pharmaceuticals (Basel). 2021;14:902. [Crossref]
42. Qian Y, Wang R, Wei W, Wang M, Wang S. Resveratrol reverses the cadmium-promoted migration, invasion, and epithelial–mesenchymal transition procession by regulating the expression of ZEB1. Hum Exp Toxicol. 2021;40(Suppl 12):S331-S338. [Crossref]
43. Choi YJ, Heo K, Park HS, Yang KM, Jeong MH. The resveratrol analog HS-1793 enhances radiosensitivity of mouse-derived breast cancer cells under hypoxic conditions. Int J Oncol. 2016;49:1479-1488.
44. Ma J, Xue M, Zhang S, Cheng L, Qian W, Duan W, et al. Resveratrol inhibits the growth of tumor cells under chronic stress via the ADRB-2-HIF-1α axis. Oncol Rep. 2019;41:1051-1058. [Crossref]
45. Zhang M, Li W, Yu L, Wu S. The suppressive effect of resveratrol on HIF-1α and VEGF expression after warm ischemia and reperfusion in rat liver. PLoS One. 2014;9:e109589. [Crossref]
46. Bahramrezaie M, Amidi F, Aleyasin A, Saremi A, Aghahoseini M, Brenjian S, et al. Effects of resveratrol on VEGF & HIF1 genes expression in granulosa cells in the angiogenesis pathway and laboratory parameters of polycystic ovary syndrome: a triple-blind randomized clinical trial. J Assist Reprod Genet. 2019;36:1701-1712. [Crossref]
47. Salaritabar A, Berindan-Neagoe I, Darvish B, Hadjiakhoondi F, Manayi A, Devi KP, et al. Targeting Hedgehog signaling pathway: Paving the road for cancer therapy. Pharmacol Res. 2019;141:466-480. [Crossref]
48. Yu P, Wang L, Tang F, Zeng L, Zhou L, Song X, et al. Resveratrol Pretreatment Decreases Ischemic Injury and Improves Neurological Function Via Sonic Hedgehog Signaling After Stroke in Rats. Mol Neurobiol. 2017;54:212-226. [Crossref]
49. Piotrowski I, Kulcenty K, Suchorska W. Interplay between inflammation and cancer. Rep Pract Oncol Radiother. 2020;25:422-427. [Crossref]
50. Mortezaee K, Najafi M, Farhood B, Ahmadi A, Shabeeb D, Musa AE. Resveratrol as an Adjuvant for Normal Tissues Protection and Tumor Sensitization. Curr Cancer Drug Targets. 2020;20:130-145. [Crossref]
51. Wang H, Jia R, Lv T, Wang M, He S, Zhang X. Resveratrol Suppresses Tumor Progression via Inhibiting STAT3/HIF-1α/VEGF Pathway in an Orthotopic Rat Model of Non-Small-Cell Lung Cancer (NSCLC). Onco Targets Ther. 2020;13:7057-7063. [Crossref]
52. Gong C, Xia H. Resveratrol suppresses melanoma growth by promoting autophagy through inhibiting the PI3K/AKT/mTOR signaling pathway. Exp Ther Med. 2020;19:1878-1886. [Crossref]
53. Wang G, Xie X, Yuan L, Qiu J, Duan W, Xu B, et al. Resveratrol ameliorates rheumatoid arthritis via activation of SIRT1-Nrf2 signaling pathway. Biofactors. 2020;46:441-453. [Crossref]
54. Tian Y, Song W, Li D, Cai L, Zhao Y. Resveratrol As A Natural Regulator Of Autophagy For Prevention And Treatment Of Cancer. Onco Targets Ther. 2019;12:8601-8609. [Crossref]
55. Farkhondeh T, Folgado SL, Pourbagher-Shahri AM, Ashrafizadeh M, Samarghandian S. The therapeutic effect of resveratrol: Focusing on the Nrf2 signaling pathway. Biomed Pharmacother. 2020;127:110234. [Crossref]
56. Aluyen JK, Ton QN, Tran T, Yang AE, Gottlieb HB, Bellanger RA. Resveratrol: potential as anticancer agent. J Diet Suppl. 2012;9:45-56. [Crossref]
57. Korniluk A, Koper O, Kemona H, Dymicka-Piekarska V. From inflammation to cancer. Ir J Med Sci. 2017;186:57-62. [Crossref]
58. Sun H, Cai H, Fu Y, Wang Q, Ji K, Du L, et al. The Protection Effect of Resveratrol Against Radiation-Induced Inflammatory Bowel Disease via NLRP-3 Inflammasome Repression in Mice. Dose Response. 2020;18:1559325820931292. [Crossref]
59. Tian X, Zhang S, Zhang Q, Kang L, Ma C, Feng L, et al. Resveratrol inhibits tumor progression by down-regulation of NLRP3 in renal cell carcinoma. J Nutr Biochem. 2020;85:108489. [Crossref]
60. Sajadimajd S, Khazaei M. Oxidative Stress and Cancer: The Role of Nrf2. Curr Cancer Drug Targets. 2018;18:538-557. [Crossref]
61. Honari M, Shafabakhsh R, Reiter RJ, Mirzaei H, Asemi Z. Resveratrol is a promising agent for colorectal cancer prevention and treatment: focus on molecular mechanisms. Cancer Cell Int. 2019;19:180. [Crossref]
62. Annaji M, Poudel I, Boddu SHS, Arnold RD, Tiwari AK, Babu RJ. Resveratrol-loaded nanomedicines for cancer applications. Cancer Rep (Hoboken). 2021;4:e1353. [Crossref]
63. Lancon A, Frazzi R, Latruffe N. Anti-Oxidant, Anti-Inflammatory and Anti-Angiogenic Properties of Resveratrol in Ocular Diseases. Molecules. 2016;21:304. [Crossref]
64. Ho Y, Li ZL, Shih YJ, Chen YR, Wang K, Whang-Peng J, et al. Integrin αvβ3 in the Mediating Effects of Dihydrotestosterone and Resveratrol on Breast Cancer Cell Proliferation. Int J Mol Sci. 2020;21:2906. [Crossref]
65. Kocyigit A, Selek S. Exogenous Antioxidants are Double-edged Swords. Bezmialem Science. 2016;2:70-75. [Crossref]
66. Valentovic MA. Evaluation of Resveratrol in Cancer Patients and Experimental Models. Adv Cancer Res. 2018;137:171-188. [Crossref]
67. Pannunzio A, Coluccia M. Cyclooxygenase-1 (COX-1) and COX-1 Inhibitors in Cancer: A Review of Oncology and Medicinal Chemistry Literature. Pharmaceuticals (Basel). 2018;11:101. [Crossref]
68. Zhang C, Liu J, Xu D, Zhang T, Hu W, Feng Z. Gain-of-function mutant p53 in cancer progression and therapy. J Mol Cell Biol. 2020;12:674-687. [Crossref]
69. Ferraz da Costa DC, Fialho E, Silva JL. Cancer Chemoprevention by Resveratrol: The p53 Tumor Suppressor Protein as a Promising Molecular Target. Molecules. 2017;22:1014. [Crossref]
70. Fu X, Li M, Tang C, Huang Z, Najafi M. Targeting of cancer cell death mechanisms by resveratrol: a review. Apoptosis. 2021;26:561-573. [Crossref]

REVIEW

DOI: 10.4274/hamidiyemedj.galenos.2022.74745

Hamidiye Med J 2022;3(2):89-91

New Insights in Aging Immunity and the BCG Vaccine

Yaşlanma Bağışıklığı ve BCG Aşısı Konusunda Yeni Görüşler

Gülbu Işıtmangil, Aysun Kaya Yıldız

University of Health Sciences Türkiye, Haydarpaşa Numune Training and Research Hospital, Clinic of Tissue Typing and Immunology, İstanbul, Türkiye

ABSTRACT

Influenza and other respiratory tract infections are the main reasons for hospital admissions and death in the elderly. It is suggested that with the strengthening of the Th1 response by means of Bacillus Calmette-Guérin (BCG) vaccination may protect them from many viral infections, including influenza and Coronavirus disease-2019 in the elderly. This review suggests that in future in the event of an outbreak, the BCG vaccine may provide a more powerful immune response against new pathogens in the elderly when applied monthly in a consecutive period of three months.

Keywords: Aging, BCG, immunity

ÖZ

Grip ve diğer solunum yolu enfeksiyonları yaşlılarda hastaneye yatış ve ölümlerin başlıca nedenleridir. Bacillus Calmette-Guérin (BCG) aşısı yoluyla Th1 yanıtının güçlendirilmesiyle, yaşlıların influenza ve Koronavirüs hastalığı-2019 dahil olmak üzere birçok viral enfeksiyondan korunmaları önerilmektedir. Bu derleme, gelecekte bir salgın durumunda, BCG aşısının birbirini takip eden üç aylık bir dönemde aylık olarak uygulandığında yaşlılarda yeni patojenlere karşı daha güçlü bir bağışıklık tepkisi sağlayabileceğini düşündürmektedir.

Anahtar Kelimeler: Yaşlanma, BCG, bağışıklık

Introduction

The immune system loses its power with age and the dimension and volume of the lymphoid organs decrease. Histologically, fibrosis, fatty tissue and the number of germinal centers increase. Wound repair and healing are insufficient. The nervous system, endocrine system and immune system lose power simultaneously. The protective mechanisms of the immune system, both congenital and acquired, progressively weaken with aging. The performances of neutrophils, monocytes, macrophages, and dendritic cells decrease leading to total decrease in chemotaxis, phagocytosis and signaling process along with ageing.

Due to a decrease in the number and function of toll-like receptors, innate immunity signals have difficulty in reaching the levels that would be sufficient to activate the adaptive immune system. The response of NK cells to inflammatory cytokines is decreased. When adaptive immunity is evaluated in the elderly, there is a decrease in the phenotypes, the number of receptors and components of T and B lymphocytes. The gradual regression and loss of function of the thymus impairs T-cell maturation. The T-cell repertoire decreases and the production of young T lymphocytes with receptor variations and heterogeneity become lower leading to a decrease in reactions against foreign antigens. The co-stimulatory signals such as B7-CD28-CTLA4 and CD40-CD154 pathways, which are important for antigen recognition and rejection/tolerance events, do not function in the elderly.

As the antigenic stimulation and memory function regulation are disrupted, antigen specific immunity decreases and the expected response to vaccinations may not occur. Also, the control of tumor production is impaired

Address for Correspondence: Gülbu Işıtmangil, University of Health Sciences Türkiye, Haydarpaşa Numune Training and Research Hospital, Clinic of Tissue Typing and Immunology, İstanbul, Türkiye
Phone: +90 216 542 32 32 E-mail: gulbu.isitmangil@sbu.edu.tr **ORCID ID:** orcid.org/0000-0003-4243-8003
Received: 29.03.2022 **Accepted:** 06.07.2022

©Copyright 2022 by University of Health Sciences Türkiye Hamidiye Faculty of Medicine. / Hamidiye Medical Journal published by Galenos Yayınevi.

due to a decrease in immune control and weakened immune regulation. Telomers in the lymphocyte nuclei shorten with aging and DNA repair thus diminishes stimulation and proliferation properties of those lymphocytes regress. The production and number of young B lymphocytes in the bone marrow decreases, while the lifespan of old B lymphocytes increases. Correspondingly, there is a decrease in the variability and quality of the antibodies produced by B lymphocytes and their affinity to the antigen. So, there is a notable weakening in the humoral immune response due to the dysregulation of B lymphocyte activation and proliferation in the elderly leading to susceptibility to infections with high mortality (1). As stated before cellular protection also weakens with immunological ageing decreasing the number of CD3+, CD4+, and CD8+ T lymphocytes beginning from the age of 45-50, together with a substantial shrinkage of the thymus gland. In summary, in the elderly Th1 cells secrete a reduced number of cytokines (IL-2, IFN-γ and TNF) where Th2 cytokine production (IL-4, IL-5, IL-10, IL-13) is higher and memory lymphocytes replace active lymphocytes which increase IL-10 production, an inhibitory cytokine.

In the elderly, these changes in the immune system suppress resistance to influenza virus infections and lead to a decrease in the protective effect of vaccines. Conversely, the response to autoantigens is augmented in the elderly, as immune system regulation is impaired and cancer and autoimmune diseases other than infections may be seen more frequently (2).

Vaccine Immunology

One of the two pathways of the immune response against vaccines is congenital innate immunity, while the other is acquired immunity. The antigenic structure comprising the vaccine is foreign to the immune system and the immune system is correspondingly stimulated. The natural immunity elements recognize the dangerous part of the vaccine antigen through the "pathogen recognition receptor". Subsequently, the vaccine antigens enter the phagocytic cells via the Toll-like receptor-like receptors in the body. Antigen presenting cells activated by mediators of the phagocytic cells present the vaccine antigen to the T lymphocytes and then antibody production is triggered by the addition of stimulation of B lymphocytes. This is the first induction of an acquired immune response by vaccine antigen and when subsequent encounters with the same antigen occur, it will result in more powerful and rapid responses by the MEMORY function of acquired immunity (3).

Normally, a vaccine triggers pathogen specific effector mechanism and provides protection against that pathogen; however, some attenuated vaccines may also provide protection against different molecules. Currently, the best example of this is the Bacillus Calmette-Guérin (BCG) vaccine.

The BCG Vaccine

The BCG vaccine is a vaccine used for the protection from tuberculosis and includes attenuated *Mycobacterium bovis* antigen (2). BCG is known to provide protection against tuberculosis in addition to providing protection against acute respiratory infections. This non-active feature of the BCG vaccine is evaluated within the concept of "trained immunity". Trained immunity can be explained as the triggering of innate immune cells to induce the reprogramming of cells. This trigger may occur by causing epigenetic and metabolic changes in the hematopoietic stem cells in the bone marrow. Netea et al. (4) were the first to propose an explanation of the mechanisms of the non-specific effect and benefit of BCG vaccination as "trained immunity" (5).

Arts et al. (6) demonstrated that BCG vaccination provided protection against experimental yellow fever virus in humans through the reprogramming of the monocytes.

BCG vaccines have been in use since 1921 and "early" and "late" vaccine strains were obtained with the passaging of three vaccines (BCG, hepatitis B and polio) administered to newborn babies. The vaccine strains have different immunologic effects and virulence. Early vaccine strains are more effective immune stimulants since they contain methoxy-mycolic acid in the cell wall in contrast to late strains. Due to the mycolic acids, high levels of IFN-gamma, myeloperoxidase and TNF-alpha are produced by macrophages. Thus, mycolic acids may lead to trained immunity. Their ability to stimulate the immune system varies, depending on the BCG vaccine strain (7).

Conversely, BCG can have an immunomodulator effect on some malignancies, as in bladder cancer. As a result of immunization with BCG, Th1 lymphocytes secreting IFN-γ and IL-2 cytokines are activated in people with bladder tumors, and the tumor shrinks as a result (8,9).

BCG and Its Effect on Trained Immunity (Effect on COVID-19)

It has been suggested that one of the reasons for the low number of cases in Asian countries during the Coronavirus disease-2019 (COVID-19) outbreak could be due to BCG immunization. We know that the BCG vaccine can protect the elderly from acute respiratory infections (6). When examined in detail, the differences between Asian countries are conspicuous and it is suggested that these differences are due to the different "BCG vaccine strains". The BCG vaccine strains used in Japan and Russia trigger trained

immunity more powerfully than the strains produced in Iran and China. Another analysis revealed that as the Russian strain was used in Türkiye it had a higher impact on trained immunity and deaths per million of the population were lower compared to others (7). It would be crucial if the validity of this hypothesis continues in a newly encountered pandemics in the future.

Protection from Pneumonia by BCG in the Elderly

In an article reported from Japan on the preventive effect of BCG vaccine against pneumonia, it was revealed that BCG strengthens the natural immunity and reduces the risk of pneumonia in elderly patients (10). The BCG vaccine has gained popularity, as pneumonia is seen frequently in COVID-19 (10).

Influenza and other respiratory tract infections are the main reasons for hospital admission and death in the elderly. Cytotoxic T-cells play a significant role in clearing the influenza virus from the lungs. Wardhana et al. (2) found a very high prevalence of acute upper rhino-pharyngo-laryngo-tracheitis (AURTI) by physical examinations of the nose, throat, and chest in a six-month observation study. In elderly individuals, scarring occurred in the areas where the BGC vaccines were administered once a month for three consecutive months, and AURTI was prevented in these individuals. The reason for this was the increased Th1 response demonstrated by the increased level of IFN-γ and the suppression of Th2 cells. It is suggested that with the strengthening of the Th1 response, protection from many viral infections, including influenza and COVID-19, can be achieved in the elderly (2).

Conclusion

In the event of an outbreak, the BCG vaccine may provide an efficient Th1 immune response in the elderly when applied monthly in a consecutive period of three months and that this may provide a powerful prevention from viral infections in the elderly.

Ethics

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: G.I., Design: G.I., A.K.Y., Literature Search: G.I., A.K.Y., Writing: G.I., A.K.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Titiz Mİ. Yaşlılarda immünite ve transplantasyon. Titiz Mİ, editor. Renal Transplantasyona Pratik Yaklaşım Humoral Sorunlar. Istanbul: Istanbul Tıp Kitabevi; 2013:59-61. [Crossref]
2. Wardhana, Datau EA, Sultana A, Mandang VV, Jim E. The efficacy of Bacillus Calmette-Guérin vaccinations for the prevention of acute upper respiratory tract infection in the elderly. Acta Med Indones. 2011;43:185-190. [Crossref]
3. Velipaşaoğlu S. Vaccine İmmünity and Factors Associated with Response to Vaccines. Osmangazi Tıp Dergisi Sosyal Pediatri Özel Sayısı. 2020;1-5. [Crossref]
4. Netea MG, Quintin J, van der Meer JW. Trained immunity: a memory for innate host defense. Cell Host Microbe. 2011;9:355-361. [Crossref]
5. Moorlag SJCFM, Arts RJW, van Crevel R, Netea MG. Non-specific effects of BCG vaccine on viral infections. Clin Microbiol Infect. 2019;25:1473–1478. [Crossref]
6. Arts RJW, Moorlag SJCFM, Novakovic B, Li Y, Wang SY, Oosting M, et al. BCG Vaccination Protects against Experimental Viral Infection in Humans through the Induction of Cytokines Associated with Trained Immunity. Cell Host Microbe. 2018;23:89-100. [Crossref]
7. Ayanoglu IC, İpekoğlu EM, Yazar V, Yılmaz IC, Gursel I, Gursel M. Could Individuals From Countries Using BCG Vaccination Be Resistant to SARS-COV-2 Induced Infections?. Turk J Immunol. 2020;8:29-36. [Crossref]
8. Böyük A, Yücel ÖB, Şanlı MÖ. Surgery in Bladder Cancer. Turkiye Klinikleri Medical Oncology - Special Topics. 2015;8:67-75. [Crossref]
9. Woldu SL, Şanlı Ö, Lotan Y. Tackling non-muscle invasive bladder cancer in the clinic. Expert Rev Anticancer Ther. 2017;17:467-480. [Crossref]
10. Noriko O. Chasing the X-factor! Why is the Japanese COVID-19 death rate low. Discuss Japan—Japan and COVID-19. 2020 Nov (Cited 2022 March): Available from: https://japan-covid19.japanpolicyforum.jp/society/pt202011112010793.html [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2022.32032

Hamidiye Med J 2022;3(2):92-98

Hepatotoxicity in Patients Using Favipiravir for COVID-19: A Retrospective Study

COVID-19 Nedeniyle Favipiravir Kullanan Hastalarda Hepatotoksisite: Retrospektif Bir Çalışma

Sinem Akkaya Işık, Burak Sarıkaya

University of Health Sciences Türkiye Hamidiye Faculty of Medicine; İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital, Clinic of Infectious Diseases and Clinical Microbiology, İstanbul, Türkiye

ABSTRACT

Background: Antimalarial drugs (hydroxychloroquine sulfate), antiretroviral drugs (lopinavir/ritonavir), and antivirals (oseltamivir, remdesivir and favipiravir) are medications used for the treatment of Coronavirus disease-2019 (COVID-19). A detailed safety analysis of favipiravir, which is used extensively in the treatment of COVID-19 in our country under pandemic conditions, is important. Investigation of the hepatotoxicity risk of favipiravir in COVID-19 patients. Our study was designed retrospectively.

Materials and Methods: Demographic characteristics, comorbid diseases and liver function test (LFT) values of the patients were retrospectively scanned and recorded. The patients were divided into two groups as died and recovered according to their results. The changes in the mean values of the LFT results and patients with different results than the reference value was evaluated according to the treatment time.

Results: Mean age of the 175 patients included in the study was 60.9±16.4 years and 122 of them were male. In the total patient population, significant ($p<0.05$) differences were found between the mean values of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and albumin on day 1 and on the days 3 and 5. As for gamma glutamyl transferase (GGT), the difference between all three consecutive measurements was significant ($p<0.05$). The change in the number of patients with abnormal, international normalized ratio (INR), alkaline phosphatase (ALP), GGT, AST, ALT, and albumin values based on treatment days was statistically significant ($p<0.05$). Analysis of this difference according to groups showed a significant difference for GGT, AST, and ALT in the survivors and for total bilirubin, ALP, INR, and albumin in the deceased ($p<0.05$).

Conclusion: It was observed that GGT, AST and ALT increased after the drug loading dose. This condition was evaluated as drug-related hepatotoxicity. However, no serious height was found in any patient to require discontinuation of favipiravir. Therefore, close monitoring for hepatotoxicity is recommended in patients treated with favipiravir, especially after the loading dose.

Keywords: Favipiravir, hepatotoxicity, COVID-19

ÖZ

Amaç: Antimalaryal ilaçlar (hidroksiklorokin sülfat), antiretroviral ilaçlar (lopinavir/ritonavir) ve antiviraller (oseltamivir, remdesivir ve favipiravir), Koronavirüs hastalığı-2019 (COVID-19) tedavisinde kullanılan ilaçlardır. Pandemi koşullarında ülkemizde COVID-19 tedavisinde yoğun kullanılan favipiravirin detaylı güvenlik analizi önemlidir. Favipiravir kullanan COVID-19 hastalarında hepatotoksisite riskinin araştırılmasıdır. Çalışmamız retrospektif olarak tasarlanmıştır.

Gereç ve Yöntemler: Hastaların demografik özellikleri, komorbid hastalıkları ve karaciğer fonksiyon test (KCFT) değerleri geriye dönük olarak taranarak kaydedildi. Hastalar sonuçlarına göre ölen ve iyileşen olarak iki gruba ayrıldı. Tedavi süresine göre KCFT sonuçlarının ortalama değerlerindeki değişimler ve referans değerden farklı sonuç veren hastalar değerlendirildi.

Bulgular: Çalışmaya alınan 175 hastanın yaş ortalaması 60,9±16,4 yıl olup 122'si erkekti. Toplam hasta popülasyonunda 1. gün ile 3. ve 5. günlerdeki aspartat aminotransferaz (AST), alanin aminotransferaz (ALT) ve albümin ortalama değerleri arasındaki fark istatistiksel olarak anlamlı idi ($p<0,05$). Gama glutamil transferazın (GGT) ardışık üç ölçümü arasındaki fark da anlamlıydı ($p<0,05$). Uluslararası normalleştirilmiş oran (INR), alkalin fosfataz (ALP), GGT, AST, ALT ve albümin değerleri anormal olan hasta sayısındaki

Address for Correspondence: Sinem Akkaya Işık, University of Health Sciences Türkiye Hamidiye Faculty of Medicine; İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital, Clinic of Infectious Diseases and Clinical Microbiology, İstanbul, Türkiye

Phone: +90 216 542 20 20 E-mail: drsinemakkaya@gmail.com **ORCID ID:** orcid.org/0000-0001-9941-2993

Received: 30.11.2021 **Accepted:** 14.02.2022

©Copyright 2022 by University of Health Sciences Türkiye Hamidiye Faculty of Medicine. / Hamidiye Medical Journal published by Galenos Yayınevi.

ÖZ

değişim, tedavi günlerine göre istatistiksel olarak anlamlıydı (p<0,05). Gruplara göre analiz yapıldığında; hayatta kalanlarda GGT, AST ve ALT, ölenlerde total bilirubin, ALP, INR ve albümin değerlerinde anlamlı farklılık tespit edildi (p<0,05).

Sonuç: İlaç yükleme dozundan sonra hastaların GGT, AST ve ALT'nin arttığı gözlendi. Bu durum ilaca bağlı hepatotoksisite olarak değerlendirildi. Ancak hiçbir hastada favipiravirin kesilmesini gerektirecek ciddi bir yükseklik bulunmamıştır. Özellikle yükleme dozundan sonra, favipiravir ile tedavi edilen hastalarda hepatotoksisite için yakın takip önerilir.

Anahtar Kelimeler: Favipiravir, hepatotoksisite, COVID-19

Introduction

Declared as a pandemic by the World Health Organization, Coronavirus disease-2019 (COVID-19) is a mostly asymptomatic or mild viral disease. Nevertheless, in 5-10% of cases, a severe clinical presentation is observed, even at times with a fatal outcome (1). For this reason, urgent treatment is required, albeit there is no definitive cure yet. Medications in use include antimalarial drugs (hydroxychloroquine sulfate), antiretroviral drugs (lopinavir/ritonavir), and antivirals (oseltamivir, remdesivir, and favipiravir) (2). Various combinations of these drugs are currently in experimental use worldwide in the treatment of COVID-19. Because of the pandemic status of COVID-19, detailed safety analyses of these drugs are of vital importance (2).

In Türkiye, Ministry of Health published a treatment guide for COVID-19, which is regularly updated online. One of the drugs recommended in this guide is favipiravir (3). Favipiravir, which was first used in the treatment of influenza, is produced through the chemical modification of a pyrosine analog. It is a selective and potent inhibitor of viral RNA polymerase. It is proven effective against Ebola and other RNA viruses, including resistant influenza strains (4).

In comparison to other treatments for COVID-19, favipiravir is an effective method and there are already studies investigating its safety and side effects (2). In Türkiye, favipiravir is administered for a total of 5 days first as a loading dose of 2x1,600 mg, followed by a maintenance dose of 2x600 mg. In our study, we aimed to investigate hepatotoxicity at these treatment doses.

Material and Methods

The study was approved by the University of Health Sciences Türkiye Hamidiye Scientific Research Ethics Committee on May 15, 2020 with the session no: 2020/5 and resolution no: 4/5. Furthermore, the study was approved by the Ministry of Health Scientific Research Platform.

Study Population and Design

The study was planned as a single-center retrospective cohort study in the University of Health Sciences Türkiye, Hamidiye Faculty of Medicine; İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital, designated as a tertiary education and research hospital from the beginning of COVID-19 outbreak. Between March 11-May 30, 2020, 202 patients who began receiving treatment with favipiravir for COVID-19 were retrospectively analyzed and 175 patients over the age 18, whose treatment was completed in 5 days, were included in our study. Since our study was planned retrospectively, patient consent was not obtained.

Data Collection

Demographic characteristics of the patients, including age, gender, and comorbidity were recorded in Microsoft Office Excel Professional.

In the study, laboratory tests providing data on liver function and hepatotoxicity were examined (5). Total bilirubin, direct bilirubin, alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), albumin, international normalized ratio (INR), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) measured on days 1, 3, and 5 of the treatment with favipiravir were recorded. Mean values of the test results for day 1, 3, and 5 were calculated. Test results were interpreted using the reference intervals recommended by our laboratory. Values falling outside these reference ranges were defined as "abnormal."

Patients were divided into two groups as deceased and survived, according to clinical outcome.

Statistical Analysis

Because of compatibility with the central limit theorem, parametric tests were used without testing normality (6). However, higher degree of deviations in the mean values measured on day 5 of the treatment required the use of the non-parametric test for ALT. In the analysis of data, especially in the statistical work of the continuous data in the scales, the mean value, standard deviation, median, quartiles, and minimum and maximum values of the characteristics were used. Frequency and percentage values were used to define

categorical variables. The means of two independent groups were evaluated with "Student's t-test" and "Mann-Whitney U test." "Repeated ANOVA test" and "Cochran's Q test" were used to compare the means for more than two dependent groups. In evaluating the relationship between categorical variables, "chi-square test" was used. Statistical significance of data was defined at $p<0.05$. In the evaluation of data, www.e-picos.com New York software and MedCalc statistics software package were used.

Results

A total of 202 patients receiving favipiravir treatment in the University of Health Sciences Türkiye Hamidiye Faculty of Medicine; İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital, were identified. Thirteen patients who had not yet completed the 5-day favipiravir treatment as the study was beginning were excluded, 9 patients who died during the favipiravir treatment, and 5 patients who were discontinued upon the decision of a physician during their follow-up. Favipiravir treatment was not discontinued due to side effects or drug interactions in any patient.

Mean age of the 175 patients included in the study was 60.9±16.4 (minimum=21 and maximum=96) years and 122 of them (69.7%) were male. In the investigation of comorbid diseases, hypertension appeared as the most frequent (75, 42.9%). No comorbidity was found in 74 (42.28%) of the patients. As for the clinical outcomes, the number of patients who died was 53 (30.3%) and the number of those who recovered was 122 (69.7%).

When other drugs used for COVID-19 treatment were examined, hydroxychloroquine sulfate use was detected in all patients. The most common choice of antibiotics for the treatment of a possible bacterial superinfection was the beta-lactam group. Among these ceftriaxone ranked the first (Table 1).

An examination of the mean values of the laboratory tests performed on study patients on day 1, 3, and 5 of the favipiravir treatment, the difference between GGT mean values on days 1, 2, and 3 was found statistically significant ($p<0.05$).

The difference between the mean values of AST, ALT, and albumin on day 1 and the mean values on days 3 and 5 were statistically significant ($p<0.05$). However, the difference between the mean values of the days 3 and 5 was not found to be statistically significant (Figure 1, Table 2).

The change in the mean values for GGT, AST, ALT, and albumin based on treatment days was significant in the recovered group of patients ($p<0.05$). For GGT, ALT, and albumin, a statistically significant difference was found between the mean values of day 1 and day 3, while there was no significant difference between the mean values of day 3 and day 5. For AST, there was a significant difference between the mean values of day 3 and day 1 and the mean values of day 3 and day 5, while no difference was found between the mean values of days 1 and 5 (Table 2).

In the group of deceased patients, significant differences ($p<0.05$) were found between the mean values of day 5 and other days for GGT and the mean values of day 1 and other days for albumin (Table 2).

Changes in the number of patients with abnormal test values were examined on the basis of treatment days. There was a statistically significant difference in the total

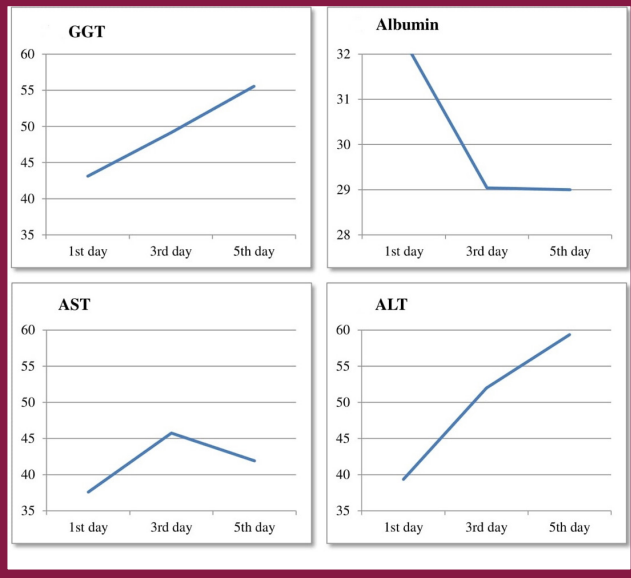

Figure 1. Change of test measurement mean values of patients using favipiravir according to treatment days. GGT: Gamma glutamyl transferase ($p<0.0001$), albumin ($p<0.0001$), AST: Aspartate aminotransferase ($p=0.03$), ALT: Alanine aminotransferase ($p=0.006$)

Table 1. Other drugs used due to COVID-19 and their distribution (n=175)

Drug	n (%)
Hydroxychloroquine sulfate	175 (100)
Lopinavir/ritonavir	28 (16)
Oseltamivir	89 (51)
Azithromycin	161 (92)
Ceftriaxone	91 (52)
Piperacillin-tazobactam	50 (29)
Moxifloxacin	10 (6)
Levofloxacin	22 (13)
Clarithromycin	16 (9)

COVID-19: Coronavirus disease-2019

patient population for ALP, GGT, INR, AST, ALT, and albumin ($p<0.05$). While there was a significant difference for GGT, AST, and ALT in the group of recovered patients, significant differences were found in the of deceased patients for total bilirubin, ALP, INR, and albumin ($p<0.05$) (Table 3).

Discussion

Research is ongoing for the specific treatment of COVID-19, a respiratory viral disease. Existing drugs, used for treating other diseases, now provide a treatment alternative (7). The side effects of these commonly used drugs gained

Table 2. Change of laboratory test measurement averages according to treatment days of patients using favipiravir

Variant (for reference, unit)		1st day MV (SD)	3rd day MV (SD)	5th day MV (SD)	p
Total bilirubin (0.1-1.2 mg/dL)	Total (n=100)	0.82 (0.95)	0.94 (1.3)	0.87 (0.82)	0.54
	Recovered (n=63)	0.76 (0.84)	0.96 (1.6)	0.72 (0.58)	0.18
	Deceased (n=37)	0.92 (1.27)	0.9 (0.74)	1.13 (1)	0.30
Direct bilirubin (0.01-0.3 mg/dL)	Total (n=102)	0.41 (0.55)	0.45 (0.74)	0.46 (0.59)	0.64
	Recovered (n=64)	0.37 (0.4)	0.42 (0.86)	0.33 (0.37	0.47
	Deceased (n=38)	0.479 (0.67	0.51 (0.5)	0.68 (0.81)	0.14
ALP (35-125 U/L)	Total (n=105)	73.45 (34.59	75.04 (46.1)	79.15 (42.15)	0.16
	Recovered (n=73)	74.09 (38.97)	77.79 (52.81)	78.67 (44.67)	0.44
	Deceased (n=32)	72 (22)	68.78 (24.36)	80.25 (36.39)	0.06
GGT (7-32 U/L)	Total (n=106)	43.11 (44.4)	49.12 (49.24)	55.55 (53.48)	**<0.0001**
	Recovered (n=74)	42.27 (46.84)	50.54 (52)	52.08 (48.59)	**0.002**
	Deceased (n=32)	45.06 (39.05)	45.84 (39.72)	63.59 (63)	**0.009**
Albumin (35-50 g/L)	Total (n:91)	32.31 (5.56)	29.04 (4.84)	29 (5.26)	**<0.0001**
	Recovered (n=55)	33.56 (5.83)	30.78 (5.11)	31.41 (4.63)	**<0.0001**
	Deceased (n=36)	30.38 (4.56)	26.38 (2.83)	25.3 (86)	**<0.0001**
INR (0.8-1.2)	Total (n=81)	1.64 (2.54)	1.6 (2)	1.52 (2)	0.53
	Recovered (n=45)	1.67 (3.1)	1.65 (2.6)	1.65 (2)	0.98
	Deceased (n=36)	1.62 (1.6)	1.52 (0.88)	1.36 (0.33)	0.48
AST (5-40 U/L)	Total (n=173)	37.58 (27.8)	45.75 (39.6)	41.91 (39.7)	**0.03**
	Recovered (n=122)	35.97 (26.35)	43.08 (34.49)	37.5 (23.94)	**0.01**
	Deceased (n=51)	41.54 (31.24)	52.32 (49.93)	52.7 (62.9)	0.33
ALT (5-40 U/L)	Total (n=172)	39.35 (31.16)	52 (49)	59.36 (70.03)	**0.006**
	Recovered (n=122)	40.16 (32.38)	55.29 (53.73)	55.49 (40.71)	**<0.0001**
	Deceased (n=50)	38.4 (28.15)	43.96 (34.39)	52.74 (77.04)	0.2

ALP: Alkaline phosphatase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma glutamyl transferase, INR: International normalized ratio, MV: Mean value, SD: Standard deviation

new importance in the circumstances of the pandemic (1,2). In our study, one of these alternative drugs used in the treatment of COVID-19, favipiravir, was investigated in terms of hepatotoxicity.

The liver has its biochemical markers that indicate its metabolic and cholestatic functions and that can be detected in the blood. Transaminases are the most important of these markers and they move outside cell as a result of increased cell membrane permeability of the hepatocyte and their levels in the blood increase (5). In our study, biochemical markers showing hepatotoxicity were examined.

The COVID-19 treatment guide published by the Turkish Ministry of Health recommends favipiravir for the treatment of patients with a need for oxygen and reimburses the costs (3). Moreover, owing to the fact that our hospital is a pandemic center and its intensive care unit bed capacity is increased, risky and poorly patients are referred to our hospital since the outbreak. For these reasons, our study includes a high number of male patients over the age of 65 and with comorbidities, all characteristics associated with the risk of a severe picture (1,8,9).

The changes in the mean laboratory values of the study patients based on treatment days were found significant for GGT, ALT, AST, and albumin. Half-lives of GGT, ALT, AST, and albumin are 26 days, 47 hours, 17 hours, and 20 days, respectively (5). The mean value of GGT, which has a long half-life, continued to increase on days 3 and 5. However, the increase in the mean values of AST and ALT, with shorter half-lives, was statistically significant on day 3, but not on day 5. In this case, it is possible to suppose that hepatotoxicity depends on the loading dose administered on day 1 of the treatment. Thus, it can be concluded that hepatotoxicity is dose-dependent for favipiravir. Low albumin levels were more common in the group of deceased patients. The rate of patients with low albumin value higher in the deceased patient group, despite its longer half-life (20 days), but the decrease in the mean value of albumin on day 3 did not continue on day 5. This was interpreted that the change in

Table 3. Change of patients with abnormal test values by treatment days

Variant		1st day n (%)	3rd day n (%)	5th day n (%)	p
Total bilurubin >1.2 mg/dL	Total (n=100)	12 (12)	16 (16)	17 (17)	0.23
	Recovered (n=63)	5 (7.9)	8 (12.7)	4 (6.3)	0.44
	Deceased (n=37)	7 (18.9)	8 (21.6)	13 (35.1)	**0.02**
Direct bilurubin >0.3 mg/dL	Total (n=102)	40 (39.2)	41 (40.2)	42 (41.2)	0.74
	Recovered (n=64)	24 (37.5)	24 (37.5)	20 (31.3)	0.58
	Deceased (n=38)	16 (42.1)	17 (44.7)	22 (57.9)	0.06
ALP >125 U/L	Total (n=105)	6 (5.7)	9 (8.6)	13 (12.4)	**0.04**
	Recovered (n=73)	3 (4.1)	5 (6.8)	7 (9.6)	0.32
	Deceased (n=32)	3 (9.3)	4 (12.5)	6 (18.7)	**0.02**
GGT >32 U/L	Total (n=106)	47 (44.3)	53 (50)	50 (47.2)	**0.009**
	Recovered (n=74)	22 (29.8)	23 (31.1)	30 (40.5)	**0.004**
	Deceased (n=32)	25 (78.1)	30 (93.7)	20 (62.5)	0.65
Albumin <32 gr/L	Total (n=91)	65 (78)	76 (87.9)	75 (85.4)	**0.04**
	Recovered (n=55)	31 (56.4)	41 (74.5)	40 (72.7)	0.35
	Deceased (n=36)	34 (94)	35 (97)	35 (97.2)	**0.02**
INR >1.2	Total (n=81)	24 (29.7)	39 (48.1)	40 (49.4)	**0.003**
	Recovered (n=45)	13 (28.9)	17 (37.8)	17 (37.8)	0.79
	Deceased (n=36)	9 (25)	12 (33.3)	23 (63.9)	**<0.0001**
AST >40 U/L	Total (n=173)	50 (28.9)	67 (38.7)	58 (33.5)	**0.03**
	Recovered (n=122)	31 (25.4)	45 (36.9)	38 (31.1)	**0.02**
	Deceased (n=51)	19 (37.3)	22 (43.1)	20 (39.2)	0.57
ALT >40 U/L	Total (n=172)	56 (32.5)	79 (45.9)	85 (49.5)	**<0.0001**
	Recovered (n=122)	41 (33.6)	59 (48.4)	66 (54.1)	**<0.0001**
	Deceased (n=50)	15 (30)	20 (40)	19 (38)	0.59

ALP: Alkaline phosphatase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma glutamyl transferase, INR: International normalized ratio

albumin value was related to the progression of the disease rather than hepatotoxicity. Chronic exposure is required for a decrease in albumin in a drug-induced hepatotoxicity (6).

The change of the number of patients with abnormal values by the day of treatment was significant for ALP, GGT, INR, AST, ALT, and albumin. This change was found significant for GGT, AST, and ALT in the group of recovered patients, whereas in the deceased group total bilirubin, ALP, INR, and albumin were also found significant. Based on these findings, it was thought that changes in AST, ALT, and GGT could be linked to drug toxicity. Total bilirubin, ALP, INR, and albumin exchange were interpreted as associated with the progression.

Being a drug long in use for influenza and gaining widespread use during the COVID-19 pandemic, favipiravir is the subject of many studies with regard to its side effects (10,11). Chen et al. (10) examined 116 patients receiving favipiravir in their comparative study on arbidol and favipiravir. In this study, liver function elevated test values were found 6 times the upper limit and elevation of transaminase was detected in 10 (8.62%) of the patients (10). In another study examining 501 cases that were given a dose lower than what our patients received, 100 patients suffered side effects. The most common side effects were elevated levels of uric acid (4.79%) and diarrhea (4.79%), while others side effects included a low neutrophil count (1.8%) and an increase in AST (1.8%) and ALT (1.6%) (12). In our study, the rates of patients with transaminase elevation were 28.9%, 38.7%, 33.5% for AST on days 1, 3, and 5, respectively; as for ALT, 32.5%, 45.9%, and 49.5%, respectively. These values are higher compared to the existing literature (10,12). In a study comparing hydroxychloroquine and favipiravir in our country, 32 patients using favipiravir were examined. As a result of the study, no statistically significant increase was found in AST, total bilirubin, direct bilirubin and ALP values in this group, while there was a statistically significant increase in ALT, LDH and GGT values (13). The fact that the drug was administered in higher doses in our study and that the reference values were accepted as limit values for the liver function test elevation criteria could account for this situation. Furthermore, our study population comprised patients with a severe clinic presentation and higher needs for oxygen. Consequently, the use of drugs that may impair liver function tests in addition to favipiravir in these patients could be considered as other factors.

There are many meta-analyses examining the efficacy and safety of favipiravir in the treatment of COVID-19 in our country and in the world. In a meta-analysis that included nine studies involving 827 patients, between the favipiravir group and the control group showed lesser odds for adverse effects in the favipiravir group but of no statistical significance ($p<0.001$) (14). Another meta-analysis showed lesser odds for adverse effect in the treatment group but of no statistical significance (odds ratio 0.69; participants=376; studie=3; I^2=88%) (15). In a meta-analysis comparing drugs used in the treatment of COVID-19, it was emphasized that the side effects caused by favipiravir, similar to our study, were mild and manageable (16).

In our study, patients had high rates of comorbidity. For this reason, patients used several different drugs for the treatment of chronic diseases. However, as our study examined the change as compared to the values on day 1, no information was included on the drugs the patients used chronically. In all patients, hydroxychloroquine sulfate and 92% azithromycin were started for the treatment of COVID-19. The risk of hepatotoxicity for hydroxychloroquine sulfate is 1-0.1%, while it is 1-2% for azithromycin. A rare side effect for oseltamivir, hepatotoxicity is seen in the range of 1-11% for lopinavir/ritonavir. There is a risk of hepatotoxicity for other drugs used, as well, but not close to the ratios found in our study (17). Consequently, the effects of the study drugs with regard to hepatotoxicity are minimal.

Study Limitations

An important limitation of our study is the liver involvement as a result of COVID-19, which requires elevated liver function tests (18). Moreover, other medications (those used for chronic conditions or other medicines used for COVID-19) may increase the hepatotoxicity risk of favipiravir. Another limitation is that in our hospital, favipiravir is administered to all patients who present with a severe course and are in need of oxygen; therefore, a control group that does not receive favipiravir from patients with the same clinical picture cannot be established.

Conclusion

It is observed that GGT, AST, and ALT, biochemical markers of hepatotoxicity, increase following favipiravir loading dose. This is considered a medication-related toxic effect. Therefore, close monitoring of patients for hepatotoxicity is recommended in a treatment with favipiravir, especially after the loading dose.

Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Türkiye Hamidiye Scientific Research Ethics Committee on May 15, 2020 with the session no: 2020/5 and resolution no: 4/5. Furthermore, the study was approved by the Ministry of Health Scientific Research Platform.

Informed Consent: Since our study was planned retrospectively, patient consent was not obtained.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.A.I., B.S., Concept: S.A.I., B.S., Design: S.A.I., Data Collection or Processing: S.A.I., B.S., Analysis or Interpretation: S.A.I., B.S., Literature Search: S.A.I., Writing: S.A.I.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Şimşek Yavuz S, Ünal S. Antiviral treatment of COVID-19. Turk J Med Sci. 2020;50:611-619. [Crossref]
2. Pilkington V, Pepperrell T, Hill A. A review of the safety of favipiravir - a potential treatment in the COVID-19 pandemic? J Virus Erad. 2020;6:45-51. [Crossref]
3. TC Sağlık Bakanlığı. COVID-19 (SARS-CoV2 Enfeksiyonu) Tedavisinde Kullanılacak İlaçlara İlişkin Bilgilendirme, Favipiravir 200 mg tablet (cited: 2020 Jun 5). Available from: https://covid19bilgi.saglik.gov.tr/depo/tedavi/ilac/FAVIPIRAVIR_200_MG_TABLET.pdf.
4. Furuta Y, Komeno T, Nakamura T. Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase. Proc Jpn Acad Ser B Phys Biol Sci. 2017;93:449-463. [Crossref]
5. Kasarala G, Tillmann HL. Standard Liver Tests. Clin Liver Dis (Hoboken). 2016;8:13-18. [Crossref]
6. Norman G. Likert scales, levels of measurement and the "laws" of statistics. Adv Health Sci Educ Theory Pract 2010;15:625-632. [Crossref]
7. Rosa SGV, Santos WC. Clinical trials on drug repositioning for COVID-19 treatment. Rev Panam Salud Publica. 2020;44:e40. [Crossref]
8. Türkiye İstatistik Kurumu. Adrese Dayalı Nüfus Kayıt Sistemi Sonuçları 2007-2019 (cited 2020 Jun 7). Available from: http://www.tuik.gov.tr/UstMenu.do?metod=temelist.
9. Ye Q, Wang B, Mao J, Fu J, Shang S, Shu Q, et al. Epidemiological analysis of COVID-19 and practical experience from China. J Med Virol. 2020;92:755-769. [Crossref]
10. Chen C, Huang J, Cheng Z, Zhang Y, Cheng Z, Wu J, et al. Favipiravir versus arbidol for COVID-19: a randomized clinical trial (serial on line) 2020 April (cited: 2020 Jun 8). Available from: https://www.medrxiv.org/content/10.1101/2020.03.17.20037432v4
11. Cai Q, Yang M, Liu D, Chen J, Shu D, Xia J, et al. Experimental treatment with Favipiravir for COVID-19: an open-label control study. Engineering (Beijing). 2020;6:1192-1198. [Crossref]
12. Tokyo, Japan: Taisho Toyama Pharmaceutical Co., Ltd. Avigan (favipiravir) tablets package insert (English translation) (serial on line) 2017 Nov (cited 2020 Jun 8). Available from: https://www.cdc.gov.tw/File/Get/ht8jUiB_MI-aKnlwstwzvw.
13. İzci-Çetinkaya F, Karagöz H, Yıldız O. Comparison of liver safety of favipiravir and hydroxychloroquine in COVID-19 treatment. Klimik Dergisi. 2020;33:235-240. [Crossref]
14. Hassanipour S, Arab-Zozani M, Amani B, Heidarzad F, Fathalipour M, Martinez-de-Hoyo R. The efficacy and safety of Favipiravir in treatment of COVID-19: a systematic review and meta-analysis of clinical trials. Sci Rep. 2021;11:11022. [Crossref]
15. Shrestha DB, Budhathoki P, Khadka S, Shah PB, Pokharel N, Rashmi P. Favipiravir versus other antiviral or standard of care for COVID-19 treatment: a rapid systematic review and meta-analysis. Virol J. 2020;17:141. [Crossref]
16. Nasir M, Perveen RA, Saha SK, Talha KA, Selina F, Islam MA. Systematic Review on Repurposing Use of Favipiravir Against SARS-CoV-2. Mymensingh Med J. 2020;29:747-754. [Crossref]
17. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury (internet). Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012. [Crossref]
18. Zhang C, Shi L, Wang FS. Liver injury in COVID-19: management and challenges. Lancet Gastroenterol Hepatol. 2020;5:428-30. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2022.07078

Hamidiye Med J 2022;3(2):99-107

Therapeutic Potential of Tannic Acid in the Management of Polycystic Ovarian Syndrome (PCOS) in Letrozole Induced Rat Model: A Histological and a Biochemical Study

Sıçanlarda Letrozol ile Oluşturulan Polikistik Over Sendromu (PKOS) Modelinde Tannik Asitin Terapötik Potansiyeli: Histolojik ve Biyokimyasal Çalışma

Fatma Gumuskaya[1,2], Tansel Sapmaz[1], Halime Tuba Canbaz[1], Sude Topkaraoglu[1], Kubra Sevgin[3], Muhammetnur Tekayev[1], Selman Aktas[4], Mahmud Esad Pence[5], Oktay Irkorucu[6]

[1]*University of Health Sciences Türkiye Hamidiye Faculty of Medicine, Department of Histology and Embryology, İstanbul, Türkiye*

[2]*Beykent University Faculty of Medicine, Department of Medical Services and Techniques, Program of Pathology Laboratory, İstanbul, Türkiye*

[3]*University of Health Sciences Türkiye Hamidiye International Faculty of Medicine, Department of Histology and Embryology, İstanbul, Türkiye*

[4]*University of Health Sciences Türkiye Hamidiye International Faculty of Medicine, Department of Biostatistics and Medical Informatics, İstanbul, Türkiye*

[5]*University of Health Sciences Türkiye, Ümraniye Training and Research Hospital, Clinic of Emergency Medicine, İstanbul, Türkiye*

[6]*University of Sharjah, College of Medicine, Department of Clinical Sciences, Sharjah, United Arab Emirates*

ABSTRACT

Background: To investigate the effects of tannic acid (TA) use on ovarian folliculogenesis, p53 expression, and serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone (T) levels in rats with polycystic ovary syndrome (PCOS) model.

Materials and Methods: A total of 18 Wistar female rats were used and divided into 3 experimental groups. Group 1 (G1, control), group 2 (G2, PCOS), group 3 (G3, PCOS+TA). Rats were induced with letrozole for 21 days to form a PCOS model. After 21 days, TA (40 mg/kg) was given by gavage for 10 days and the rats were sacrificed on the 10th day. PCOS formation was evaluated by daily estrous cycle follow-up. Hematoxylin & eosin and p53 immunohistochemical staining was performed on ovaries. Serum FSH, LH, and T levels were determined by ELISA. Data were analyzed with the One-Way ANOVA test and Kruskal-Wallis H test. $P<0.05$ was considered statistically significant.

Results: The number of cystic follicles was significantly increased in the PCOS group compared to the TA treatment group ($p<0.05$). The number of primary follicles was significantly increased in the TA treatment group ($p<0.001$). No significant change was observed in the number of primordial, secondary, and Graaf follicles between the experimental groups. A significant increase in LH and T was observed in the PCOS group ($p<0.05$). The increase in LH has significantly decreased TA administered rats ($p<0.05$). Although it was not significant, serum FSH level was increased in the PCOS+TA group. No immunoreactivity was detected in p53 staining in experimental groups.

Conclusion: TA can decrease cystic follicle formation and increase primary follicle formation in PCOS. Also, it can regulate the hormonal expression of serum LH, FSH, and T in PCOS-modeled rats.

Keywords: Letrozole, PCOS, rat, tannic acid

Address for Correspondence: Tansel Sapmaz, University of Health Sciences Türkiye Hamidiye Faculty of Medicine, Department of Histology and Embryology, İstanbul, Türkiye

Phone: +90 505 565 69 28 E-mail: sapmaz.tansel@gmail.com **ORCID ID:** orcid.org/0000-0002-7820-5837

Received: 29.03.2022 **Accepted:** 20.04.2022

©Copyright 2022 by University of Health Sciences Türkiye Hamidiye Faculty of Medicine. / Hamidiye Medical Journal published by Galenos Yayınevi.

ÖZ

Amaç: Polikistik over sendromu (PKOS) modeli oluşturulmuş sıçanlarda tannik asit (TA) kullanımının ovaryan folikülojenez, p53 ekspresyonu ve serum lüteinizan hormon (LH), folikül uyarıcı hormon (FSH) ve testosteron (T) seviyeleri üzerindeki etkilerini araştırmaktır.

Gereç ve Yöntemler: Toplam 18 Wistar dişi sıçan kullanıldı ve 3 deney grubuna ayrıldı: Grup 1 (G1, kontrol), grup 2 (G2, PKOS), grup 3 (G3, PKOS+TA). Sıçanlara, PCOS modeli oluşturmak için 21 gün boyunca letrozol verildi. Yirmi bir gün sonra TA (40 mg/kg) 10 gün boyunca gavaj yoluyla verildi ve sıçanlar 10. günde sakrifiye edildi. PKOS oluşumu günlük östrus döngüsü takibi ile değerlendirildi. Over dokusunda hematoksilen & eosin ve p53 immünohistokimyasal boyamaları yapıldı. Serum FSH, LH ve T seviyeleri ELISA ile belirlendi. Veriler One-Way ANOVA testi ve Kruskal-Wallis H testi ile analiz edildi. P<0,05 istatistiksel olarak anlamlı kabul edildi.

Bulgular: PKOS grubunda kistik folikül sayısı TA tedavi grubuna göre anlamlı olarak arttı (p<0,05). TA tedavi grubunda primer folikül sayısı anlamlı olarak arttı (p<0,001). Deney grupları arasında primordial, sekonder ve Graaf foliküllerinin sayısında önemli bir değişiklik gözlenmedi. PKOS grubunda LH ve T'de anlamlı bir artış gözlendi (p<0,05). TA uygulanan sıçanlarda LH seviyesinde önemli ölçüde azalma gözlendi (p<0,05). PKOS+TA grubunda anlamlı olmasa da serum FSH düzeyi arttı. Deney gruplarında p53 boyamasında immünoreaktivite saptanmadı.

Sonuç: TA, PKOS'de kistik folikül oluşumunu azaltabilir ve primer folikül oluşumunu artırabilir. Ayrıca TA, PKOS modelli sıçanlarda serum LH, FSH ve T'nin hormonal ekspresyonunu düzenleyebilir.

Anahtar Kelimeler: Letrozol, PKOS, sıçan, tannik asit

Introduction

Polycystic ovary syndrome (PCOS) is a complex metabolic disease characterized by chronic anovulation and hyperandrogenism, affecting 5-10% of women of reproductive age (1). It is a health problem with many clinical findings such as anovulation, amenorrhea, oligomenorrhea, menstrual irregularities, dysfunctional uterine bleeding, and hirsutism. More serious long-term risks are developing infertility, endometrial hyperplasia, endometrial cancer, dyslipidemia, coronary artery disease, and possible breast cancer (2). The chance of implantation of the embryo in the uterus of PCOS patients decreases (3). Abnormal endocrine and paracrine factors, metabolic dysfunctions, and changes in the microenvironment during folliculogenesis cause failure in the oocyte maturation and embryonic development in women with PCOS (4,5). Impaired oocyte sufficiency in PCOS is inevitably associated with abnormal follicle development. In PCOS patients, insulin resistance and paracrine disorder of growth factors, including transforming growth factor-β disrupt the follicular environment. This changes the relationship between granulosa cells and oocytes and damages the cytoplasm of the oocyte and the nucleus maturation (6,7,8,9,10).

PCOS is characterized by abnormal secretion of gonadotropins (11). PCOS also is an increased synthesis of steroid hormones by the ovaries (11). In particular, the secretion of LH and T is increased in women with PCOS. The ratio of luteinizing hormone/follicle-stimulating hormone (LH/FSH) is elevated with PCOS, it results in the ovaries producing more androgens. Furthermore, in PCOS patients, the levels of insulin and insulin-like growth factors are elevated. This situation eventually increases the production of androgens by follicular theca cells and enhances LH function (12). Apoptosis and the cellular proliferation ratio are essential for the normal physiological function of the body. However, this balance is disturbed in PCOS patients. Some factors are related to this syndrome, including the tumor regulatory gene, *p53* (13,14).

The most common secondary metabolites in plants are polyphenols and tannins are among the most widely studied phenolic compounds (15). Tannins are classified into hydrolyzed tannins and condensed tannins. Tannic acid (TA) is a kind of hydrolyzed tannin (16). Tannins have been reported to promote mammalian ovulation rate and embryo development (17). Also, TA prevents damage to biological molecules in the body by inhibiting hydroxyl radicals (18). TA has been accepted by the Food and Drug Administration as a safe chemical (19). TA can interact directly with biomacromolecules such as collagen, gelatin, and albumin due to its ability to form electrostatic interactions, hydrogen bonds, and hydrophobic interactions (20). Recently, it has been shown that TA can be used as an anti-cancer chemical in various animal cells (21). It has been shown that TA increases plasma androgen and estrogen in both pikas and root voles (22). It has been reported that low concentrations of tannins promote the *in vitro* embryo development of the mouse oocyte (23). However, there are very limited studies related to explaining why tannins could affect the reproduction of animals.

In the literature, there is no study demonstrating the effect of TA on experimental PCOS models in rats. The present study was carried out in an attempt to elucidate the effects of TA on letrozole-induced PCOS modelled rats with three main objectives. First, to determine whether TA administration in the PCOS model is correlated with

circulating LH, FSH, and T concentrations, and to determine whether TA can regulate the p53 expressions, and third, to determine whether TA can improve ovarian architecture and folliculogenesis in PCOS modeled rats.

Material and Methods

The experimental study was performed in the laboratory of the Hamidiye Experimental Animals Production and Research Laboratory, University of Health Sciences Türkiye. Ethical approval of the project was obtained from the Hamidiye Animal Experiments Local Ethics Committee, University of Health Sciences Türkiye (no: 2019-03/01). All experimental animal procedures were performed following standard ethical guidelines. Scientific Research Projects Unit, University of Health Sciences Türkiye (no: 2019/071) supported this study.

PCOS Model and Experimental Groups

A total of 18 female adult Wistar Albino rats weighing 170-250 g and 7-9 weeks old were used. The rats were drunk into city water and fed with standard pellet feed in a room with a 12-h light 12 hours dark photoperiod. The temperature was 21-23 °C. Rats in the estrus phase were determined by vaginal cytology follow-up. From the 10th day until the last day of the experiment, vaginal smear analysis was performed every day. The estrus cycle was followed by methylene blue staining. While examining the preparations, the result was reached by taking the cell structures as a reference (24). Weight control of experimental animals was done every day.

Letrozole was dissolved in 1 mL 0.5% carboxymethyl cellulose (CMC). All agents were given the gavage method. The rats were randomly divided into 3 prospective groups as follows:

The control group (n=6): The group in which 0.5% CMC was given every day during the experimental period.

PCOS group (n=6): The group in which 1 mg/kg/day letrozole® (FL24873, Cymıt Químıca S.L., Spain) was given for 21 days then 1 mL of distilled water was given for 10 days.

PCOS+TA group (n=6): The group in which 1 mg/kg/day letrozole® was given for 21 days and then 40 mg/kg/day TA was given for 10 days.

Histopathological Assessment

At the end of the experiment, 75 mg/kg ketasol and 10 mg/kg xylazine anesthesia were applied intraperitoneally to all rats. All rats were sacrificed by exsanguination and blood samples were collected via intracardiac way. The left ovaries of all the groups were isolated for histopathological assessment. The ovaries were fixed with 10% formaldehyde for pathological examination and embedded in paraffin blocks after standard tissue processing. The blocks were sectioned with a thickness of 5 µm. Preparations were stained with HE and evaluated under light microscopy in terms of ovarian follicle number.

To assess ovarian architecture and follicle number, three sections of ovarian tissue were selected. Blinded observers evaluated the sections independently. Primordial, primary, secondary, graafian, and cystic follicles were counted as suggested by Souza et al. (25). Follicles were counted: As primordial follicle; surrounded by thin single layers of follicular epithelial cells surrounding the oocyte, primary follicle; the single or multilayered prismatic epithelium surrounding the oocyte, secondary follicle; follicle in which the oocyte is lined with more than two granulosa cells with a small antrum, graafian follicle; follicle containing a single large antrum, in which the oocyte is surrounded by some cumulus cells, cystic follicles; follicles with degrading granulosa cells in the thin layer of granulosa cells (26). The stained tissue samples were observed by a light microscope (Zeiss, Scope.A1, Germany).

Immunohistochemistry (IHC) Staining of p53

Apoptosis of the cells was measured by IHC staining of p53 [Novocastra™ Liquid Mouse Monoclonal Antibody, p53 protein (DO-7), United Kingdom]. Deparaffinized ovary slides were rehydrated and incubated in citrate buffer for antibody retrieval. The lides were incubated in 3% of H_2O_2 to quench the endogenous peroxidase activity. After blocking, slides were incubated with a p53 antibody at 4 °C overnight. Then, slides are incubated with biotinylated secondary antibodies, streptavidin-horseradish peroxidase, and DAB (3,3′-diaminobenzidine) on the next day. Counterstaining was performed with a hematoxylin solution for 5 min, and then slides were mounted after dehydration. The stained tissue samples were observed by a light microscope (Zeiss, Scope.A1, Germany).

Biochemical Assessment

The blood was centrifuged at 12000 rpm for 10 minutes, serum samples were stored at -80 °C. Serum T (E0259Ra, BT Lab, Korea), FSH (EA0015Ra, BT Lab., Korea), and LH (E0179Ra, BT Lab., Korea) concentrations were measured by ELISA kits. The standard procedures were followed during ELISA measurements.

Statistical Analysis

Statistical analysis of the data was performed with SPSS 25.0 (Statistical Package for the Social Sciences) program. The distribution of variables was analyzed using the Shapiro-Wilk test and normally distributed variables

were reported as mean ± standard deviation and non-normally distributed variables were reported as median (minimum-maximum). Normally distributed variable groups were examined by the One-Way ANOVA test and non-normally distributed variable groups were examined with the Kruskal-Wallis H test. Within-group differences were examined using the Bonferroni correction. Different time measures of dependent variables were examined using the Repeated Measure ANOVA test. P<0.05 was considered statistically significant.

Results

Evaluation of the Estrous Cycle

The experiment was successful in all rats. Estrus cycles were determined by methylene blue staining (Figure 1A). Regular estrus cycles were observed in the vaginal smear preparations of rats belonging to the control (G1) group. Vaginal smear preparations from rats belonging to the PCOS group (G2) exhibited an irregular estrous cycle; mostly in the estrus phase. It was observed that the estrous cycle was regular in the PCOS+TA group.

Evaluation of Body Weights

In all groups, the weight gain increased as the rat age increased weekly (Figure 1B). Accelerated weight gain was observed in the PCOS model group compared to the control and PCOS+TA group.

Light Microscopy

Normal ovarian morphology was exhibited in the control group in the histopathological analysis. Many cystic follicles were present in the PCOS group. The ovarian tissue of letrozole-induced PCOS rats depicted cystic expansion, enlarged cystic sinus follicles, and decreased granulosa cell layers (Figure 2). The improved ovarian architecture was detected in the PCOS+TA group as indicated by the decreasing number of cystic follicles and an increasing number of graafian follicles in comparison to the PCOS group. It has been observed that treatment with TA reduced the theca cell layer, increased granulosa cell layers, and improved mature follicles in ovaries.

Table 1 shows the data of the primordial follicle, secondary follicle, Graaf follicle, and cystic follicle numbers. The number of cystic follicles was significantly increased in the PCOS group compared to the TA treatment group ($p<0.05$). The number of primary follicles was significantly increased in the TA treatment group compared to the PCOS group ($p<0.001$). No significant change was observed in the number of primordial, secondary, and Graaf follicles between the experimental groups. Light microscopic results supported the analysis of follicular count (Figure 2).

IHC Staining of p53

Apoptosis was determined by assessing the expression of p53 (Figure 3). The p53 immunoreactivity in the ovaries of all animals belonging to the control and experimental groups was examined.

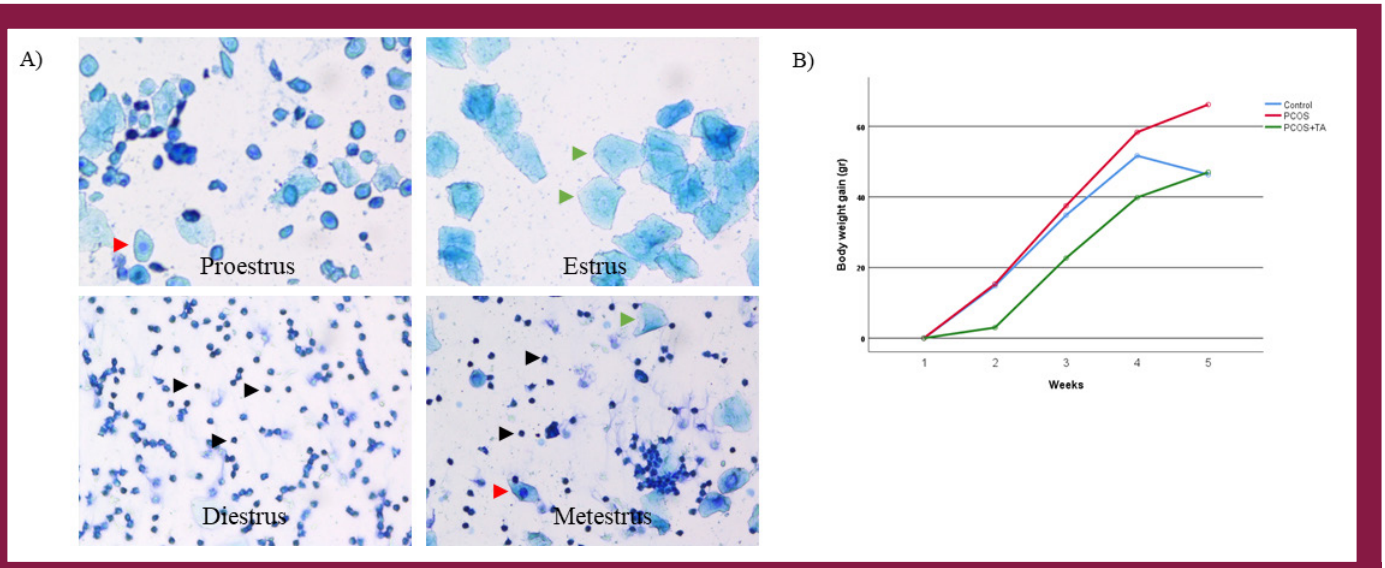

Figure 1. Methylene blue staining of vaginal smears. Nucleated epithelial cells (red arrowhead) are observed in the proestrus stage. Anucleated cornified cells (green arrowhead) are observed in the estrus phase. Leukocytes (black arrowhead) are observed only in the diestrus phase. Nucleated epithelium, anucleated corniphoe cells, and leukocytes are observed in the metestrus phase. The light microscope. 10X magnification (A). Change of rats' weight over weeks (B)

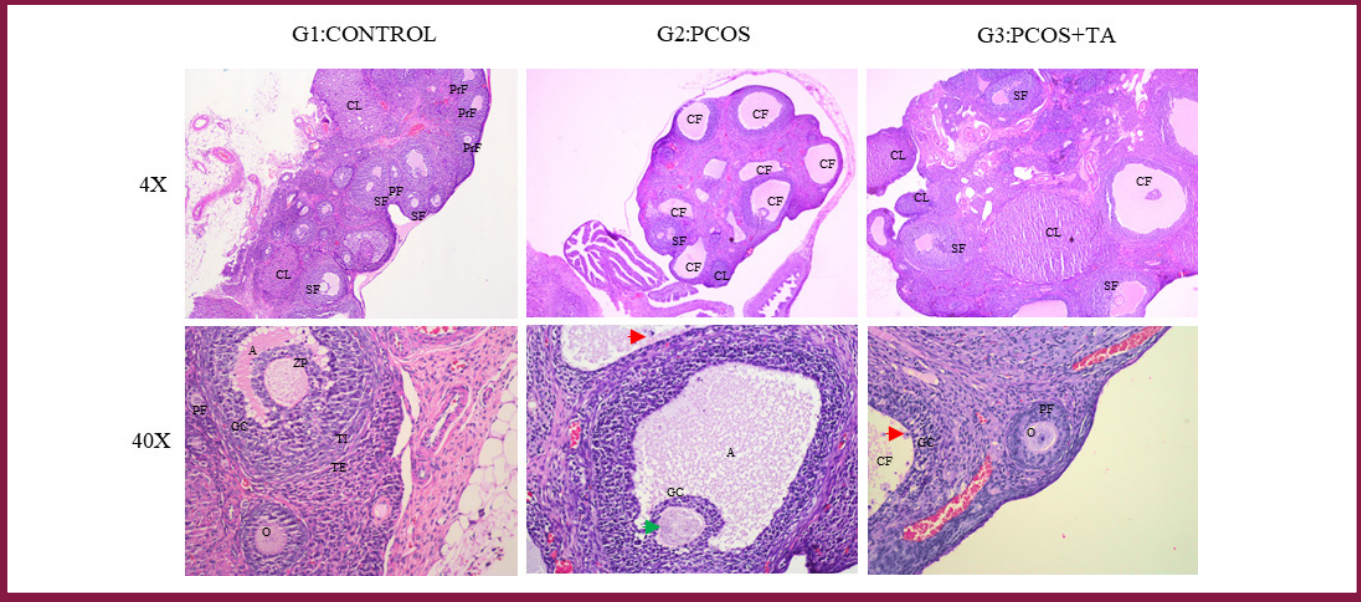

Figure 2. Histological assessment of the ovary. The control group (G1) with normal ovarian cortex structure. The cortex area exhibited the normal developing follicles with arranged PFs (G1, 4X). Theca and granulosa cells depicted regular and intact organization (G1, 40X). No the degeneration was observed in the oocyte and surrounding zona pellucida cells (G1, 40X). PCOS and the PCOS+TA groups exhibited cystic follicles. PCOS group displayed a few corpus luteum compared to other groups (G2, 4X). Also, layers of granulosa cells were decreased (G2, 40X). Apoptotic granulosa cells with pycnotic nuclei (red arrowhead), a marker of follicle atresia, were observed in the follicles in the PCOS and PCOS+TA groups. At the same time, degeneration of oocyte and zona pellucida was detected (green arrowhead) (G2, 40X). In the Tannic Acid treatment group, oocyte and surrounding zona pellucida were normal and intact in some follicles (G3, 40X). Cystic follicles were also present in the TA group (G3, 4X). G1: Control, G2: PCOS, G3: PCOS+TA.

A: Antrum, PF: Primordial follicle, SF: Secondary follicle, GC: Granulosa cell, O: Oocyte, TC: Theca cells, PrF: Primary follicle, PCOS: Polycystic Ovary syndrome, CF: Cystic follicle, TA: Tannic acid. Light microscope. HE, 4X, 40X magnification

Table 1. Mean ± SD of the primordial follicle, graaf follicle, and cystic follicle values and median (min-max) of the secondary follicle, and corpus luteum values in control, PCOS, and TA treated rats

		Statistic		p	Difference
Primordial follicle	Control	Mean ± SD	4.5±2.81	0.168[a]	No difference
	PCOS		1.50±1.51		
	PCOS+TA		3.50±2.07		
Primary follicle	Control	Median (min-max)	5.50 (5-10)	0.008[b]	PCOS+TA>PCOS
	PCOS		3.50 (2-6)		
	PCOS+TA		7 (6-12)		
Secondary follicle	Control	Median (min-max)	3 (2-9)	0.454[b]	No difference
	PCOS		2 (2-5)		
	PCOS+TA		3.5 (1-7)		
Graaf follicle	Control	Mean ± SD	2±1.41	0.086[a]	No difference
	PCOS		0.83±0.76		
	PCOS+TA		1.67±0.81		
Cystic follicle	Control	Median (min-max)	2.5 (1-4)	0.017[b]	PCOS>PCOS+TA
	PCOS		3.5 (3-4)		
	PCOS+TA		2 (1-3)		

PCOS: Polycystic ovary syndrome, SD: Standard deviation, TA: Tannic acid, [a]: ANOVA, [b]: Kruskal-Wallis H test

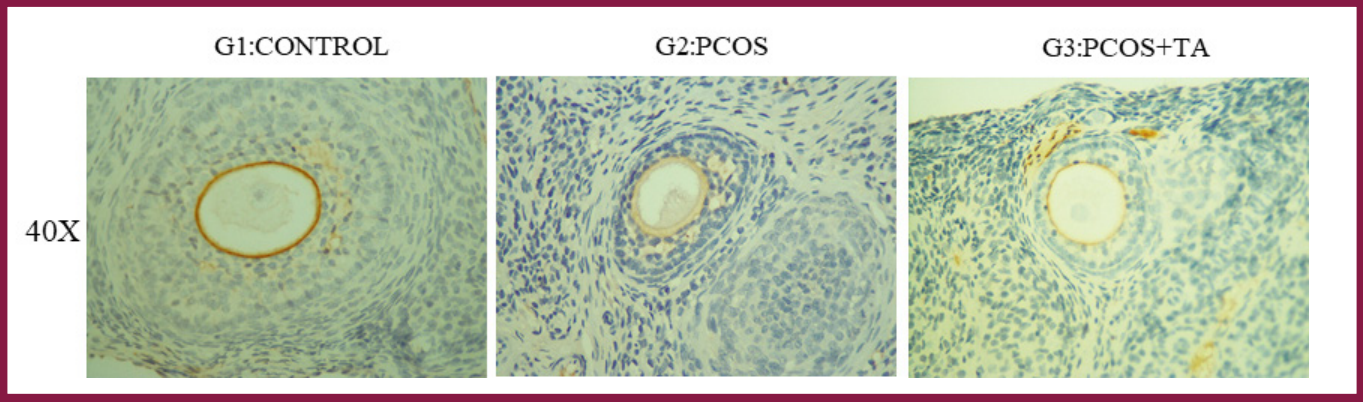

Figure 3. P53 immunohistochemical staining. There was a false positive in some areas but overall, no staining was detected. No difference was observed between the groups. G1: Control, G2: PCOS, G3: PCOS+TA. The light microscope. 40X magnification

PCOS: Polycystic ovary syndrome, TA: Tannic acid

Table 2. Examination of biochemical parameters (LH, FSH, T) between experimental groups

		Median (min-max)	Test statistic	p	Difference
Testosterone	Control	78.65 (40.97-107.98)	0.6351	0.042[a]	PCOS>control
	PCOS	131.3 (68.78-149)			
	PCOS+TA	102.62 (25-120)			
FSH	Control	1.83 (1.53-2.63)	1.31	0.519[a]	No difference
	PCOS	1.94 (1.80-2.17)			
	PCOS+TA	2.10 (1.78-2.23)			
LH	Control	1.04 (0.57-1.24)	11.789	0.003[a]	PCOS>control PCOS>PCOS+TA
	PCOS	1.76 (1.67-1.93)			
	PCOS+TA	1.07 (1.00-1.64)			

PCOS: Polycystic ovary syndrome, TA: Tannic acid, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, T: Testosterone, [a]: Kruskal-Wallis H test

Immunoreactivity was not observed in any of the control and experimental groups. In the PCOS group, p53 staining in the nuclear areas was determined below 1%.

Biochemical Assessment

Table 2 shows the results of the biochemical parameters. In terms of hormone LH and T levels, the PCOS group had a significant increase compared to the control group ($p<0.05$). The increase in LH was significantly decreased in the PCOS+TA group ($p<0.05$). Although it was not significant, serum FSH level was increased in the PCOS+TA group.

Discussion

PCOS is a disease characterized by an androgen increase in the ovaries, ovulation disorder, and cystic structures in the ovaries (27). This endocrine disease affects 4-8% of women of reproductive age. 40% of women with PCOS are infertile due to anovulation disorder (28). The diagnosis of PCOS is based on many criteria. PCOS treatment, causes, genetic transmission, roles of related genes, and gene regulation have not been fully elucidated (29). TA is an antioxidant compound, contains polyphenolic compounds, so it can mitigate various conditions of oxidative stress (30). In this study, we hypothesize that the administration of TA might have a potential curative effect against PCOS-modelled rat ovaries. To test this hypothesis, serum LH, FSH, and T levels, the number of follicles, and p53 expression were examined. Our study showed that 40 mg/kg TA treatment significantly decreased the number of cystic follicles. Also, although TA treatment cannot fully improve the p53 expression, it can control the hormone profile, the ovarian follicular cell architecture, and folliculogenesis in PCOS.

Since anovulation is one of the symptoms of PCOS, the estrous cycle of rats was followed by a vaginal smear examination. It has been previously reported that letrozole-

treated rats lost their regular estrus period (31). In our study, it was determined that the letrozole-administered rats exhibited an irregular estrous cycle, which depicts successful PCOS modeling. It was determined that letrozole caused prolongation of the estrus cycle in rats, apoptosis in granulosa cells, thinning of the membranes, and an increase in the number of antral follicles. In our study, it was determined that rats administered letrozole showed irregular and prolonged cycles and TA administration regulated the estrus cycle. These findings depicted that TA administration may control and regulate the estrus cyclicity in PCOS ovaries. Our finding are in accord with the literature (32).

Although obesity is not the only cause of PCOS, it plays a role in the pathophysiology of this disease. Glucose intolerance and insulin resistance have been found to be other causes of obesity in women with PCOS (33). In this study, the body weights of the rats were evaluated weekly, it was determined that the weights of the PCOS group increased in comparison to the control group. It was statistically determined that body weight gain decreased in the TA treatment group. These findings are consistent with previous studies in the literature (34).

To our knowledge, this study is the first report demonstrating a significant depletion in cystic follicles in the PCOS model by TA treatment. 40 mg/kg TA treatment decreased the elevated number of cystic follicles significantly in comparison to PCOS ($p<0.05$). These indicate the potential therapeutic effects of the TA treatment on the maintenance of folliculogenesis in the PCOS model (Table 1). Although no statistically significant difference was found, it was determined that the number of primordial follicles in the ovary was decreased in rats in the PCOS group. It was observed that the follicle reserve could not be preserved in the PCOS group due to the decrease in primordial follicular tissue, but it was preserved in the TA group. Furthermore, the number of cystic follicles was increased significantly in PCOS rats ($p<0.05$). The elevated number of cystic follicles is caused by hyperandrogenism (13). Since granulosa cells cannot transform into luteinized granulosa cells due to low FSH in PCOS, progesterone production and ovulation do not occur. A slowdown or hesitation at any stage of this mechanism causes the follicle to remain in the form of millimetric cysts in the ovaries. Therefore, with the monthly recurrence of these events, the number of millimetric cysts in the ovary increase (35). The effects of TA might be due to its potent antioxidant and anti-inflammatory properties. An increase in the number of primary follicle counts was significant in TA administered group compared to the PCOS group ($p<0.001$) (Table 1). In the PCOS group, the primary follicles were decreased; pointed out that some of these follicles in the folliculogenesis process have undergone atresia or formed secondary follicles. This idea is consistent with the increased secondary follicle counts, especially in the PCOS group. In histopathological evaluation, letrozole-induced PCOS rats depicted cystic expansion, enlarged cystic sinus follicles, and decreased granular cell layers. These features are consistent with the pathological changes observed in human PCOS ovaries (Figure 2).

In PCOS patients, the secretion of LH and T is increased on the other hand FSH is decreased. When the LH/FSH ratio is elevated, the ovaries preferentially synthesize androgens. Therefore, PCOS-diagnosed women have higher levels of LH and T (36). In PCOS, ovulation does not occur due to low FSH and there is no progesterone production since granulosa cells cannot transform into luteinized granulosa cells (37). In this study, a significant increase in LH and T was observed in the PCOS group ($p<0.05$) (Table 2). The increase in LH significantly decreased TA administered rats ($p<0.05$) (Table 2). Serum T level was also decreased in TA administered rats. This reveals that TA can control the serum LH and T levels in PCOS modelled rats. No significant difference was observed in serum FSH levels. In humans, a decrease in serum FSH levels is seen in PCOS patients, but no statistical difference was observed in rats (38). Serum FSH level was increased in the PCOS+TA group. This suggests that TA can control FSH release in PCOS. Although it was not significant, a slight increase in serum FSH levels can be explained by an increase in FSH level at the very beginning of the prolonged estrus phase in PCOS rats (38).

The largest receptor group of apoptotic signals are molecules that have important roles in the cell cycle. One of the proteins that is best understood to control apoptosis is the p53 protein (39). The balanced ratio of cellular apoptosis and proliferation is disturbed in patients with PCOS. There is evidence that the cell's cycle-related tumor regulatory gene, p53, is responsible for the etiology of PCOS (13). The p53 has been demonstrated to play a role in granulosa cell apoptosis. Altered expression of p53 may contribute to the possible enhanced follicular resistance to apoptotic signals in PCOS (40). Cui et al. (41) investigated whether follicular dysplasia is associated with the regulation of apoptosis in ovarian granulosa cells. They showed that while the S phase was longer in the PCOS group in comparison to the control group, the G2/M phase was shorter in length and the cells in the PCOS group were subjected to apoptosis (41). In our study, an IHC study was applied to show the apoptotic cells in granulosa cells and to determine the effect of the p53 expression pattern on the pathogenesis of PCOS at the protein level. However, a significant immunopositivity could not be determined in immunohistochemical light microscopy examinations of p53 (Figure 3). This indicated

that PCOS follicles do not go atrophy. Also indicated that TA treatment cannot induce the apoptotic p53 mechanism.

Study Limitations

The present study has certain limitations. The circulating LH, FSH, and T concentrations were measured as the final outcome, but it is not clear whether TA treatment concentration would successfully regulate pregnancy. Furthermore, the present study failed to show whether low or high TA treatment is more important in controlling hormonal disturbances. Also, as this was an animal study, the results could not be considered linear for humans.

Conclusion

We observed that TA can alleviate histological disturbances in the PCOS model of rats. To the best of our knowledge, we reported for the first time that TA may be an effective and good alternative therapeutic agent in ameliorating PCOS histological disturbances and hormonal regulation. However, further studies are needed to confirm the safety of TA and its proper dose for PCOS treatment.

Ethics

Ethics Committee Approval: Research Laboratory, University of Health Sciences Türkiye. Ethical approval of the project was obtained from the Hamidiye Animal Experiments Local Ethics Committee, University of Health Sciences Türkiye (no: 2019-03/01).

Informed Consent: All experimental animal procedures were performed following standard ethical guidelines.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.G., M.T., S.A., Concept: F.G., T.S., H.T.C., S.T., K.S., O.I., Design: F.G., T.S., H.T.C., S.T., K.S., O.I., Data Collection or Processing: F.G., K.S., M.T., M.E.P., Analysis or Interpretation: F.G., K.S., S.A., Literature Search: F.G., T.S., H.T.C., S.T., K.S., O.I., Writing: T.S., H.T.C., S.T., K.S., O.I.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Franks S. Polycystic ovary syndrome. N Engl J Med. 1995;333:853-861. [Crossref]
2. Speroff L. Fritz MA. Eds. Clinical gynecologic endocrinology and infertility. Lippincott Williams & Wilkins; 2005. [Crossref]
3. Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, et al. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. Curr Biol. 2004;14:1296-1302. [Crossref]
4. Franks S, Roberts R, Hardy K. Gonadotrophin regimens and oocyte quality in women with polycystic ovaries. Reprod BioMed Online. 2003;6:181-184. [Crossref]
5. Wood JR, Dumesic DA, Abbott DH, Strauss JF 3rd. Molecular abnormalities in oocytes from women with polycystic ovary syndrome revealed by microarray analysis. J Clin Endocrinol Metab. 2007;92:705-713. [Crossref]
6. Copp J, Manning G, Hunter T. TORC-specific phosphorylation of mammalian target of rapamycin (mTOR): phospho-Ser2481 is a marker for intact mTOR signaling complex 2. Cancer Res. 2009;69:1821-1827. [Crossref]
7. Yaba A, Bianchi V, Borini A, Johnson J. A putative mitotic checkpoint dependent on mTOR function controls cell proliferation and survival in ovarian granulosa cells. Reprod Sci. 2008;15:128-138. [Crossref]
8. Franks S, Stark J, Hardy K. Follicle dynamics and anovulation in polycystic ovary syndrome. Hum Reprod Update. 2008;14:367-378. [Crossref]
9. Elia E, Sander V, Luchetti CG, Solano ME, Di Girolamo G, Gonzalez C, et al. The mechanisms involved in the action of metformin in regulating ovarian function in hyperandrogenized mice. Mol Hum Reprod. 2006;12:475-481. [Crossref]
10. Sander VA, Facorro GB, Piehl L, Rubín de Celis E, Motta AB. Effect of DHEA and metformin on corpus luteum in mice. Reproduction. 2009;138:571-579. [Crossref]
11. Braillon A. Polycystic ovary syndrome. N Engl J Med. 2005;352:2756-2757. [Crossref]
12. Johansson J, Stener-Victorin E. Polycystic ovary syndrome: Effect and mechanisms of acupuncture for ovulation induction. Evid Based Complement Alternat Med. 2013;2013:762615. [Crossref]
13. Naseri L, Khazaei MR, Khazaei M. Synergic effect of bee pollen and metformin on proliferation and apoptosis of granulosa cells: Rat model of polycystic ovary syndrome. J Food Biochem. 2022;46:e13635.
14. Aksoy S, Armutcu F, Yiğitoglu MR. Using of caffeic acid phenetil ester (CAPE) the active substance of propolis in some neurologic disease and emergency. Spatula DD. 2011;1:37-42. [Crossref]
15. D'Archivio M, Filesi C, Di Benedetto R, Gargiulo R, Giovannini C, Masella R. Polyphenols, dietary sources and bioavailability. Ann Ist Super Sanita. 2007;43:348-361. [Crossref]
16. Zhao ZJ, Wang DH. Effects of diet quality on energy budgets and thermogenesis in Brandt's voles. Comp Biochem Physiol A Mol Integr Physiol. 2007;148:168-177. [Crossref]
17. Min BR, Fernandez JM, Barry TN, McNabb WC, Kemp PD. The effect of condensed tannins in Lotus corniculatus upon reproductive efficiency and wool production in ewes during autumn. Anim Feed Sci Technol. 2001;92:185-202. [Crossref]
18. Sen HM, Ozkan A, Guven M, Akman T, Aras AB, Sehitoglu I, et al. Effects of Tannic Acid on the Ischemic Brain Tissue of Rats. Inflammation. 2015;38:1624-1630. [Crossref]
19. Ahmad T. Reviewing the tannic acid mediated synthesis of metal nanoparticles. Journal of Nanotechnology. 2014;11:18-39. [Crossref]
20. Sahiner N, Sengel SB. Tannic acid decorated poly (methacrylic acid) micro and nanoparticles with controllable tannic acid release and antioxidant properties. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2016;508:30-38. [Crossref]
21. Sahiner N, Sagbas S, Aktas N, Silan C. Inherently antioxidant and antimicrobial tannic acid release from poly (tannic acid) nanoparticles with controllable degradability. Colloids Surf B Biointerfaces. 2016;142:334-343. [Crossref]
22. Dai X, Zou ZZ, Zhang TT, Jiang W, Wei WH, Yang SM. The effect of tannic acid on the gonad hormones in plateau pikas (Ochotona curzoniae) and root voles (Microtus oeconomus). Acta Theriol Sinica. 2011;31:278-283. [Crossref]

23. Spinaci M, Volpe S, De Ambrogi M, Tamanini C, Galeati G. Effects of epigallocatechin-3-gallate (EGCG)-on in vitro maturation and fertilization of porcine oocytes. Theriogenology. 2008;69:877-885. [Crossref]
24. Ekambaram G, Sampath Kumar SK, Joseph LD. Comparative Study on the Estimation of Estrous Cycle in Mice by Visual and Vaginal Lavage Method. J Clin Diagn Res. 2017;11:AC05-AC07. [Crossref]
25. Souza AZ, Fonseca AM, Izzo VM, Clauzet RM, Salvatore CA. Ovarian histology and function after total abdominal hysterectomy. Obstet Gynecol. 1986;68:847-849. [Crossref]
26. Wang F, Wang S, Zhang Z, Lin Q, Liu Y, Xiao Y, et al. Defective insulin signaling and the protective effects of dimethyldiguanide during follicular development in the ovaries of polycystic ovary syndrome. Mol Med Rep. 2017;16:8164-8170. [Crossref]
27. Sirmans SM, Pate KA. Epidemiology, diagnosis, and management of polycystic ovary syndrome. Clin Epidemiol. 2013;6:1-13. [Crossref]
28. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr Rev. 2012;33:981-1030. [Crossref]
29. Witchel SF, Oberfield SE, Peña AS. Polycystic Ovary Syndrome: Pathophysiology, Presentation, and Treatment With Emphasis on Adolescent Girls. J Endocrine Soc. 2019;3:1545-1573. [Crossref]
30. Varanka Z, Rojik I. VarankaI, Nemcsók J, Ábrahám M. Biochemical and morphological changes in crap liver following ex- posure to copper sulfate and tannic acid. Comparative Biochemistry and Physiology Part C Toxicology & Pharmacology 2001;128:467-478. [Crossref]
31. Jahan S, Abid A, Khalid S, Afsar T, Qurat-Ul-Ain, Shaheen G, et al. Therapeutic potentials of Quercetin in management of polycystic ovarian syndrome using Letrozole induced rat model: a histological and a biochemical study. J Ovarian Res. 2018;11:26. [Crossref]
32. Osuka S, Nakanishi N, Murase T, Nakamura T, Goto M, Iwase A, et al. Animal models of polycystic ovary syndrome: A review of hormone-induced rodent models focused on hypothalamus-pituitary-ovary axis and neuropeptides. Reprod Med Biol. 2018;18:151-160. [Crossref]
33. Aydos A, Oztemur Y, Dedeoglu BG. Polycystic ovary syndrome and molecular approaches. Turk Hij Den Biyol Derg. 2016;73:81-88. [Crossref]
34. Sun L, Ji C, Jin L, Bi Y, Feng W, Li P, et al. Effects of Exenatide on metabolic changes, sexual hormones, inflammatory cytokines, adipokines, and weight change in a DHEA-treated rat model. Reprod Sci. 2016;23:1242-1249. [Crossref]
35. Goodarzi MO, Azziz R. Diagnosis, epidemiology, and genetics of the polycystic ovary syndrome. Best Pract Res Clin Endocrinol Metab. 2006;20:193-205. [Crossref]
36. Hoseinpour MJ, Ghanbari A, Azad N, Zare A, Abdi S, Sajadi E, et al. Ulmus minor bark hydro-alcoholic extract ameliorates histological parameters and testosterone level in an experimental model of PCOS rats. Endocr Regul. 2019;53:146-153. [Crossref]
37. Joseph-Horne R, Mason H, Batty S, White D, Hillier S, Urquhart M, et al. Luteal phase progesterone excretion in ovulatory women with polycystic ovaries. Hum Reprod. 2002;17:1459-1463. [Crossref]
38. Miller BH, Takahashi JS. Central circadian control of female reproductive function. Front Endocrinol (Lausanne). 2014;4:195. [Crossref]
39. Bennett MR, Littlewood TD, Schwartz SM, Weissberg PL. Increased sensitivity of human vascular smooth muscle cells from atherosclerotic plaques to p53-mediated apoptosis. Circ Res. 1997;81:591-599. [Crossref]
40. Homburg R, Amsterdam A. Polycystic ovary syndrome--loss of the apoptotic mechanism in the ovarian follicles? J Endocrinol Invest. 1998;21:552-557. [Crossref]
41. Cui X, Jing X, Wu X, Bi X, Liu J, Long Z, et al. Abnormal expression levels of BMP15/Smad1 are associated with granulosa cell apoptosis in patients with polycystic ovary syndrome. Mol Med Rep. 2017;16:8231-8236. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2022.24633

Hamidiye Med J 2022;3(2):108-117

Upgrade Rates of DCIS, Intraductal Papilloma, and the Other High-risk Breast Lesions

DCIS, İntraduktal Papillom ve Diğer Yüksek Riskli Meme Lezyonlarında Upgrade Oranları

Emine Yıldırım[1], Sibel Bektaş[2], Muhammed Özdemir[1], Ahenk Karagülle[2], Ahmet Muzaffer Er[1], Neşe Uçar[3]

[1]*University of Health Sciences Türkiye, Gaziosmanpaşa Training and Research Hospital, Clinic of General Surgery, İstanbul, Türkiye*

[2]*University of Health Sciences Türkiye, Gaziosmanpaşa Training and Research Hospital, Clinic of Pathology, İstanbul, Türkiye*

[3]*University of Health Sciences Türkiye, Gaziosmanpaşa Training and Research Hospital, Clinic of Radiology, İstanbul, Türkiye*

ABSTRACT

Background: In benign breast lesions such as intraductal papilloma (IDP), atypical hyperplasia (AH), flat epithelial atypia (FEA) and lobular carcinoma *in situ* (LCIS), there is a 3-20% risk of upgrade to invasive or *in situ* breast cancer following excision. The aim of this study was to determine the upgrade rates for high-risk breast lesions (HRBL), which were diagnosed by core needle biopsy (CNB), to invasive or *in situ* breast carcinoma, and to determine to upgrade rates for ductal carcinoma *in situ* (DCIS) to invasive breast carcinoma in the second group. In addition, we investigated in which patient groups these rates are higher.

Materials and Methods: It was planned to include all female patients who had undergone surgical procedures following the determination of IDP, AH, FEA, LCIS, or DCIS after CNB under ultrasonographic guidance between April 2014 and August 2020. As there were no patients diagnosed with pure LCIS with biopsy, this was not included in the analysis. Patients were excluded from the study if more than 6 months had elapsed between CNB and excision, or if they had a history of breast cancer or radiotherapy. Demographic data, radiological findings and histopathological results were collected retrospectively from the hospital records.

Results: A total of 123 patients with diagnosis following CNB were evaluated. The diagnoses were IDP in 70.7% of patients, AH in 8.9%, FEA in 4.9%, and DCIS in 15.5%. The upgrade rates for invasive breast cancer were 30%, 0%, 16.7%, and 31.6%. The upgrade rates for DCIS were calculated as 3.5% in IDP, 45.5% in AH, and 0% in FEA. Especially, in IDP group upgrade was seen more at older ages, and when there were more than 2 two papilloma ($p<0.05$). The upgrade risk for DCIS after excision was 31.6%.

Conclusion: The upgrade risk for HRBL was found to vary between 5.8% and 45.5%, and the upgrade risk for DCIS after excision was 31.6%. In patients with HBRL; older ages, the presence of a multifocal lesion, a palpable mass, and radiological-histopathological discordance were seen to be risk factors for upgrade.

Keywords: Intraductal papilloma, atypical ductal hyperplasia, proliferative lesions with atypia, upgrade, breast cancer

ÖZ

Amaç: Benign meme lezyonları arasında bulunan intraduktal papillom (IDP), atipik hiperplaziler (AH), flat epitelyal atipi (FEA) ve lobüler karsinoma *in situ* (LCIS) eksizyon sonrası invaziv veya *in situ* meme kanseri için %3-20 arasında değişen upgrade riski taşır. Çalışmada amacımız kalın iğne biyopsisi (CNB) sonrası tanı konan yüksek riskli meme lezyonlarının (HRBL) invaziv veya *in situ* meme kanserine upgrade oranlarını belirlemek ve ikinci bir grup olarak duktal karsinoma *in situ* (DCIS) için invaziv meme kanserine upgrade oranlarını bulmaktı. Ek olarak hangi hastalarda upgrade oranlarının daha yüksek olduğunu araştırdık.

Gereç ve Yöntemler: Nisan 2014-Ağustos 2020 tarihleri arasında ultrasonografi eşliğinde CNB yapıldıktan sonra IDP, AH, FEA, LCIS veya DCIS saptanıp cerrahi işlem uygulanan tüm kadın hastaların dahil edilmesi planlanmıştır. Ancak biyopsi ile pür LCIS tanılı hasta olmadığı için bu hastalar çalışma dışı bırakılmıştır. Ayrıca CNB ile eksizyon arasında 6 aydan uzun süre geçen, meme kanseri veya radyoterapi öyküsü olan hastalar çalışmaya dahil edilmemiştir. Demografik, radyolojik ve histopatolojik veriler retrospektif olarak hasta dosyalarından toplanmıştır.

Address for Correspondence: Sibel Bektaş, University of Health Sciences Türkiye, Gaziosmanpaşa Training and Research Hospital, Clinic of Pathology, İstanbul, Türkiye
Phone: +90 532 707 31 53 E-mail: sibel.bektas@sbu.edu.tr **ORCID ID:** orcid.org/0000-0003-0248-9869

Received: 30.11.2021 **Accepted:** 01.05.2022

©Copyright 2022 by University of Health Sciences Türkiye Hamidiye Faculty of Medicine. / Hamidiye Medical Journal published by Galenos Yayınevi.

ÖZ

Bulgular: CNB sonrası tanı alan 123 hasta çalışmaya dahil edildi. Hastaların %70,7'si IDP, %8,9'u AH, %4,9'u FEA ve %15,5'i DCIS tanılı idi. İnvaziv meme kanseri için upgrade oranları sırası ile %2,3, %0, %16,7 ve %31,6 bulundu. DCIS için upgrade IDP'de %3,5, AH'de %45,5, FEA'da %0 olarak hesaplandı. Özellikle IDP grubunda ileri yaşlarda ve 2'den fazla iki papillom olduğunda upgrade daha fazla görüldü (p<0,05). DCIS'nin eksizyon sonrası upgrade riski %31,58 olarak bulundu.

Sonuç: Yüksek riskli meme lezyonlarının upgrade riski %5,75-45,45 arasında değişirken, DCIS'nin eksizyon sonrası upgrade riski %31,58 olarak bulundu. HBRL'li hastalarda; ileri yaş, multifokal lezyon varlığı, ele gelen kitle ve radyolojik-histopatolojik uyumsuzluğun upgrade için risk faktörleri olduğu görüldü.

Anahtar Kelimeler: İntraduktal papillom, atipik duktal hiperplazi, proliferatif atipili lezyonlar, upgrade, meme kanseri

Introduction

In 1985, breast lesions were first grouped as non-proliferative, proliferative without atypia, and proliferative atypical lesions, and the malignancy risks were reported with upgrade rates for each of these lesions (1). Within these, the group with an increased risk of breast cancer and showing an upgrade to invasive cancer or ductal carcinoma *in situ* (DCIS) after excision is accepted as high-risk breast lesions (HRBL). The HRBL group includes intraductal papilloma (IDP), atypical ductal and lobular hyperplasia (ADH-ALH), flat epithelial atypia (FEA), and lobular carcinoma *in situ* (LCIS) (2).

IDPs, which are a specific group within proliferative breast lesions without atypia, have upgrade rates of 5-20%. It has also been reported that this risk increases if a palpable mass is present, if a mass >1 cm is determined on mammography (MG) or ultrasonography (US), or if there are more than 5 papillomas (3).

FEA is a borderline lesion which is a precursor to low-grade invasive and *in situ* cancers, which is grouped in the proliferative atypical breast lesions (4). It is generally diagnosed with core needle biopsy (CNB) applied to microcalcification seen as suspicious on MG. Although the risk is lower in FEA patients when there is compatibility between radiological and histopathological diagnoses, upgrade rates varying from 9.6% to 15% have been reported. ADH or ALH accompanying the lesion increases the upgrade risk (4).

Atypical hyperplasia often emerges as proliferative atypical breast lesions in breast biopsies. The upgrade rates range from 10-20%, and have been reported as <3% in ALH diagnosed incidentally with radiology compatible with the histopathological diagnosis. Treatment with surgical excision following biopsy is recommended for ADH. However, although there are those who recommend surgical excision for ALH, in cases diagnosed incidentally where radiological and histopathological diagnoses are not compatible, follow-up is thought to be sufficient (5,6,7). The risk of the development of ipsilateral or contralateral breast cancer in atypical hyperplasia is 3-4-fold greater than that in the general population (1).

DCIS is a proliferation of ductal epithelial cells in the breast that does not extend beyond the basement membrane and has no evidence of invasion (8). In contrast to all other high-risk lesions, in DCIS, subsequent invasive breast cancer develops in the same breast and same quadrant, and therefore DCIS is accepted as a precursor lesion (8,9). Invasive cancer can be seen after surgical excision in 10-20% of patients with DCIS diagnosed with CNB (10).

The aim of this study was to determine the upgrade rates for HRBL and DCIS diagnosed with CNB in our hospital through evaluation of excisional biopsy results and to demonstrate in which patient group the upgrade risk is higher.

Material and Methods

Case Selection and Study Design

The study included all female patients determined by breast mass or suspicious microcalcification who underwent surgical excision following a diagnosis of IDP, FEA, ADH, ALH, or DCIS as a result of CNB between April 2014 and August 2020. Calcifications that are irregular in size or shape or are tightly clustered together, are called suspicious calcifications. No patient was diagnosed with pure LCIS with biopsy between the defined dates. All the LCIS diagnoses were accompanying invasive cancer, so patients with a diagnosis of LCIS were not included in the study. Other exclusion criteria were i) A period of more than 6 months between CNB and excision, and ii) A history of breast cancer or radiotherapy. Data were retrieved from the medical records and a retrospective review was made of patient age, physical examination, breast US, MG, and magnetic resonance imaging (MRI) findings, and CNB and pathology results.

Imaging Targets and Biopsy Techniques

Breast imaging included US, MG, and MRI methods. Each image was evaluated according to the Breast Imaging

Reporting and DATA System (BIRADS) classification (11). Following the evaluation, CNB was taken from BIRADS 3, 4, and 5 lesions, which were >2 cm, showed growth in follow-up and were developing morphological changes. Biopsy procedures were applied under sterile conditions after local anesthesia with 1% lidocaine underwent the patient. Core biopsies were obtained under USG guidance; at least 6 pieces were collected using a 14-G automatic core-biopsy needle (Geotek Inc., Ankara, Türkiye). After the procedure, the samples were placed into tubes containing 10% formalin and sent for pathological analysis.

Histopathological Assessement

HRBL were defined as IDP, FEA, ADH, and ALH. The histopathological evaluation was defined as follows;

IDP; a lesion formed of branching structures with fibrovascular cores covered by benign epithelial cells (12).

FEA; is by definition flat, i.e. lacks architectural atypia, but has low-grade cytologic atypia (13).

ADH; epithelial proliferation formed from a neoplastic cell population similar in appearance to low-grade DCIS and limited to the breast ductal-lobular system at low volume or dimensions (<2 canals involved or total size ⩽0.2 cm) (14).

ALH; small uniform neoplastic cell proliferation showing loose cohesion similar to LCIS involving <50% of acini in the terminal ductal lobular unit (9).

DCIS; neoplastic proliferation of breast ductal epithelial cells limited to the ductal-lobular system without evidence of invasion from the basal membrane to the stroma (15).

Upgrade was defined as the diagnosis of DCIS or invasive cancer after excision of the lesion initially defined as benign or atypical on CNB. Excisional biopsy was performed on patients with HRBL after CNB and the upgrade rates were determined according to this result.

Statistical Analysis

Data obtained in the study were analyzed statistically using SPSS vn. 21 software. The conformity of continuous data to normal distribution was assessed with the Shapiro-Wilk test. Parametric tests were applied to data showing normal distribution and non-parametric tests to data not showing normal distribution. In the comparisons of two independent groups, the Student's t-test or the Mann-Whitney U test were used, and for more than two groups, One-Way ANOVA or the Kruskal Wallis test. In the analysis of categorical data, the chi-square test and the Fisher's Exact test were used. A value of $p<0.05$ was accepted as statistically significant. Thus, the data obtained from clinicopathological and imaging findings were evaluated with the chi-square test. The descriptive features of data such as age and lesion size were evaluated with the One-Way ANOVA and Kruskal-Wallis tests.

Results

Between April 2014 and August 2020, a total of 206 patients were diagnosed with BIRADS 3, 4, or 5 lesions on breast imaging. It was seen that 123 patients were operated on for a diagnosis of HRBL. The CNB results of those patients were 87 IDP, 6 FEA, 11 ADH and 19 DCIS. Imaging of the patients was performed with US, MG, or MRI and all the patients were evaluated according to the BIRADS categories.

When the groups were evaluated ultrasonographically, there was seen to be a regular contoured solid mass in more of the IDP group (36.9%), most of the patients with a normal image (50%) were in the FEA group, and in most patients in the ADH and DCIS groups, there was an appearance of a solid mass (56.4%, 62.5%, respectively). A statistically significant difference was determined between the groups in respect of the US imaging characteristics ($p=0.029$). When we look at the mammographic imaging features, asymmetric density was higher in the patients in the IDP group, while Asymmetric density and microcalcification were more common in the DCIS group ($p>0.05$).

When the distribution according to the BIRADS characteristics was examined, most lesions in all groups were BIRADS 3, followed by BIRADS 4 in the IDP, FEA, and ADP groups, and BIRADS 5 lesions in the DCIS group. A statistically significant difference was determined between the groups in respect of BIRADS distribution ($p<0.001$). There was discordance between radiological and histopathological diagnoses in 62.1% of IDPs, 50% of FEAs, 90.9% of ADHs, and in 68.3% of DCISs. This difference between the groups was statistically significant ($p=0.029$). The demographic, clinicopathological, and imaging findings are shown in Table 1.

There were 87 patients in the IDP group, the mean lesion size was 16.07±10.76 mm, and the mean age was 47.24±12.82 years. A palpable mass was detected in 53 of the patients (60.9%), 33 patients (37.9%) had radiological-histopathological discordance, and 82 patients (94.3%) had a single lesion. The upgrade rate for IDP was found to be 5.8%, upgrade was seen more at older ages, and when there were more than 2 papillomas ($p<0.05$). Although not statistically significant, the presence of a palpable mass was determined in all lesions with upgrade in the IDP group and concordance was lower in the group with upgrade (with upgrade 40%; without upgrade 62.2%). In 2 cases that developed invasive ductal cancer (IDC) in this group, there was seen to be discordance. These results were found to be clinically significant. There was atypia in the biopsy sample of one (1.2%) patient diagnosed with IDP.

Table 1. Demographic, clinicopathologic and imaging findings in the patients

Characteristic	Lesions								Total		
	IDP		FEA		ADH		DCIS				
	Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD		p[1]
Age (year)	47.24±12.82		43.5±8.48		57.36±9.36		50±13.18		12.7±8.48		0.058
Lesion size (mm)	16.07±10.76		14.17±10.15		18.51±14.26		25.55±24.68		17.66±14.3		0.764[a]
	n	%	n	%	n	%	n	%	n	%	p[2]
Ultrasonography (n=120)											
Irregular contour solid mass	26	31.0	2	33.3	3	27.3	6	31.6	37	30.8	**0.029***
Regular contour solid mass	31	36.9	0	0.0	3	27.3	4	21.1	38	31.7	
Irregular contour cystic mass	15	17.9	1	16.7	2	18.2	1	5.3	19	15.8	
Regular contour cystic mass	3	3.6	0	0.0	1	9.1	1	5.3	5	4.2	
Ductal ectasia	5	6.0	0	0.0	0	0.0	0	0.0	5	4.2	
Normal imaging	4	4.8	3	50.0	2	18.2	7	36.8	16	13.3	
Mammography (n=94)											
Asymmetric density	19	29.7	2	40.0	1	12.5	1	5.9	23	24.5	0.124
Asymmetric density and mass	18	28.1	1	20.0	3	37.5	5	29.4	27	28.7	
Asymmetric density microcal and mass	5	7.8	0	0.0	1	12.5	1	5.9	7	7.4	
Asymmetric density microcalcification	7	10.9	1	20.0	3	37.5	8	47.1	19	20.2	
Normal imaging	15	23.4	1	20.0	0	0.0	2	11.8	18	19.1	
MRI (n=55)											
Mass	21	70.0	0	0.0	3	50.0	5	31.3	29	52.7	0.095
No mass	9	30.0	3	100.0	3	50.0	11	68.8	26	47.3	
BIRADS (n=123)											
3	32	36.8	2	33.3	4	36.4	2	10.5	40	32.5	**<0.001***
4	53	60.9	3	50.0	6	54.5	10	52.6	72	58.5	
5	2	2.3	1	16.7	1	9.1	7	36.8	11	8.9	
Palpable mass											
Present	53	60.9	5	83.3	7	63.6	12	63.2	77	62.6	0.75
Absent	34	39.1	1	16.7	4	36.4	7	36.8	46	37.4	
Concordance											
Present	33	37.9	3	50.0	1	9.1	2	10.5	39	31.7	**0.029***
Absent (discordance)	54	62.1	3	50.0	10	90.9	17	89.5	84	68.3	

p[1]: One-Way ANOVA ([a]Kruskal-Wallis test), p[2]: Chi-square test, *: Statistically significant (p<0.05), IDP: Intraductal papilloma, FEA: Flat epihtelial atypia, ADH: Atypical ductal hyperplasia, DCIS: Ductal carcinoma *in situ*, BIRADS: Breast imaging reporting and DATA system, Concordance: Mention of imaging-histologic concordance, SD: Standard deviation

There were a total of 6 patients in the FEA group. The mean age of the patients was 43.5±8.48 years, and the mean lesion size was 14.17±10.15 mm. While two patients (40%) had multifocal lesions, 5 patients (83.3%) had palpable masses. In the FEA group, there was one lesion with upgrade (16.7%), and there was radiological-histopathological concordance in this lesion, which was also multifocal and there was a palpable mass.

The ADH group comprised 11 patients. The mean age of the patients was 57.36±9.36 years, and the mean lesion size was 18.51±14.26 mm. Two patients (18.2%) had multifocal lesions, seven patients (63.6%) had palpable masses, and 2 patients (18.2%) had radiological-histopathological discordance. The upgrade rate for ADH was found to be 45.5% and all the patients showed upgrade to IDC. Palpable mass was detected in 3 of 5 (60%) patients who were upgraded. Evaluations were made in this group (p>0.05).

Nineteen patients included in the study were diagnosed with DCIS. The mean lesion size in these patients was 25.55±24.68 mm, and the age was 50±13.18 years. In this

group, 12 (63.2%) of the patients had palpable masses, 2 patients (10.5%) had radiological-histopathological discordance and 7 (36.8%) had multifocality. In the examination of DCIS, a palpable mass was determined in 83.3% of the lesions with upgrade and in 53.9% of the lesions without upgrade. The upgrade rate for DCIS was found to be 31.6%. In patients with DCIS, the mean age of the patients in the upgraded group was lower. Although the findings were clinically significant, they were not statistically significant. The findings are shown in Table 2.

The total upgrade rate for HRBL was 10.6%. The IDC upgrade rate for DCIS was found to be 31.6%. For IDP, the DCIS upgrade rate was 3.5% and the IDC upgrade rate was 2.3%. For FEA, the IDC upgrade rate was calculated as 16.7% and for ADH, the DCIS upgrade rate was 45.5%. The upgrade rates for HRBL and DCIS are shown in Table 3. Pathological samples of the patients who developed an upgrade are shown in Figures 1 and 2.

Table 2. Characteristics of groups with upgrade and no upgrade in high-risk breast lesions and DCIS

Intraductal papilloma							
	Total (n=87)		**Upgrade (n=5)**		**No upgrade (n=82)**		
Characteristic	**Mean ± SD**		**Mean ± SD**		**Mean ± SD**		**p[1]**
Age (year)	47.24±12.82		60.2±7.29		46.45±12.68		0.019*
Lesion size (mm)	16.07±10.76		20.4±20.83		15.81±10.01		0.985[a]
	n	**%**	**n**	**%**	**n**	**%**	**p[2]**
Palpable mass							
Present	53	60.92	5	100	48	58.54	0.065[a]
Absent	34	39.08	0	0	34	51.46	
Concordance							
Present	54	62.07	2	40	51	62.2	1.00[a]
Absent (discordance)	33	37.93	3	60	31	37.8	
Number of lesion							
Single	82	94.25	2	40	80	97.56	<0.001*
2-5	3	03.44	2	40	1	1.22	
>5	2	02.29	1	20	1	1.22	
Flat epithelial atypia							
	Total (n=6)		**Upgrade (n=1)**		**No upgrade (n=5)**		
Characteristic	**Mean ± SD**		**Mean ± SD**		**Mean ± SD**		**p[1]**
Age (year)	43.5±8.48		41		44±9.38		NC
Lesion size (mm)	14.17±10.15		5		16±10.17		NC
	n	**%**	**n**	**%**	**n**	**%**	**p[2]**
Palpable mass							
Present	5	83.33	1	100	4	80	1.00[a]
Absent	1	16.66	0	0	1	20	
Concordance							
Present	3	50	1	100	2	40	1.00[a]
Absent (discordance)	3	50	0	0	3	60	
Multifocal							
Present	2	40	1	100	1	20	0.333[a]
Absent	4	60	0	0	4	80	
Atypical ductal hyperplasia							
	Total (n=11)		**Upgrade (n=5)**		**No upgrade (n=6)**		
Characteristic	**Mean ± SD**		**Mean ± SD**		**Mean ± SD**		**p[1]**

Table 2. continued

Age (year)	57.36±9.36		57.2±10.06		57.5±9.71		0.961
Lesion size (mm)	18.51±14.26		26.6±16.89		11.77±7.65		0.177[a]
	n	**%**	**n**	**%**	**n**	**%**	**p[2]**
Palpable mass							
Present	7	63.64	3	60	4	66.67	1.00[a]
Absent	4	36.36	2	40	2	33.33	
Concordance							
Present	9	81.82	4	80	5	83.33	1.00[a]
Absent (discordance)	2	18.18	1	20	1	16.67	
Multifocal							
Present	2	18.18	2	40	0	0	0.182[a]
Absent	9	81.82	3	60	6	100	
Ductal carcinoma *in situ*							
	Total (n=19)		**Upgrade (n=6)**		**No upgrade (n=13)**		
Characteristic	**Mean ± SD**		**Mean ± SD**		**Mean ± SD**		**p[1]**
Age (year)	50±13.18		46.83±15.56		51.46±12.34		0.493
Lesion size (mm)	25.55±24.68		20.42±15.98		27.92±28.07		0.701[a]
	n	**%**	**n**	**%**	**n**	**%**	**p[2]**
Palpable mass							
Present	12	63.16	5	83.33	7	53.85	0.333[a]
Absent	7	36.84	1	16.67	6	46.15	
Concordance							
Present	17	89.47	6	100	11	84.62	1.00[a]
Absent (discordance)	2	10.53	0		2	15.38	
Multifocal							
Present	7	36.84	3	50	4	30.77	0.617[a]
Absent	12	63.16	3	50	9	69.23	

p[1]: Student's t test ([a]Mann-Whitney U test), p[2]: Chi-square test ([a]Fisher's Exact test) NC: Not calculated, SD: Standard deviation, *: Statistically significant (p<0.05)

Discussion

Knowing the risk of upgrade in breast lesions is very important in decision-making for follow-up or excision. With the developments in imaging methods over time, there has started to be more detailed evaluation of lesions, and in parallel with this, decisions have become clearer in determining the lesions from which biopsy will be taken and those for which subsequent excision is planned.

IDP, FEA, ADH, ALH, and LCIS are high-risk lesions in respect of upgrade after excision. It has been reported that an upgrade to invasive cancer can be seen in up to 20% of DCIS (16,17).

In studies in literature, MacColl et al. (18) reported the upgrade risk for IDP as 12% (8.3% DCIS and 3.3% invasive breast cancer. According to that study, the risk is greater in the group with high BIRADS, in the older ages group, in lesions that contain calcifications and in lesions >5 mm in size (18).

Qui et al. (19) determined an upgrade rate of 11.1% for IDP and reported that in all the cases with upgrade to invasive cancer, there was accompanying atypia in the biopsy and no upgrade in benign papilloma.

Han et al. (20) found the upgrade risk to be 0.8% for IDP without atypia and stated that conservative follow-up may be sufficient in solitary lesions that are thought to be benign with CNB and which do not show clinically suspicious characteristics, and in patients without concurrent contralateral breast cancer.

Table 3. High risk breast lesions upgrade rate

Tru-cut biopsy	Excision						Total upgrade rate (DCIS+invasive Ca)	
	No upgrade			Invasive Ca				
	n		%	n		%	n	%
DCIS (n=19)	13		68.42	6		31.58	6	31.58
	No upgrade		DCIS		Invasive Ca			
	n	%	n	%	n	%	n	%
IDP (n=82)								
Atypia	1	1.15	0		0		0	0
No atypia	81	93.1	3	3.45	2	2.3	1	5.75
Total	**82**	**94.25**	**3**	**3.45**	**2**	**2.3**	**7**	**5.75**
FEA (n=6)								
Atypia	1	16.67	0	0	0	0	0	0
No atypia	4	66.67	0	-	1	16.67	1	16.67
Total	**5**	**83.33**	**0**	**0**	**1**	**16.67**	**1**	**16.67**
ADH (n=11)	6	54.54	5	45.45	0	0	-	45.45
All patient (IDP, FEA, ADH) (n=104)	93	89.42	8	7.69	3	2.88	11	10.58

IDP: Intraductal papilloma, FEA: Flat epithelial atypia, ADH: Atypical ductal hyperplasia, DCIS: Ductal carcinoma *in situ*

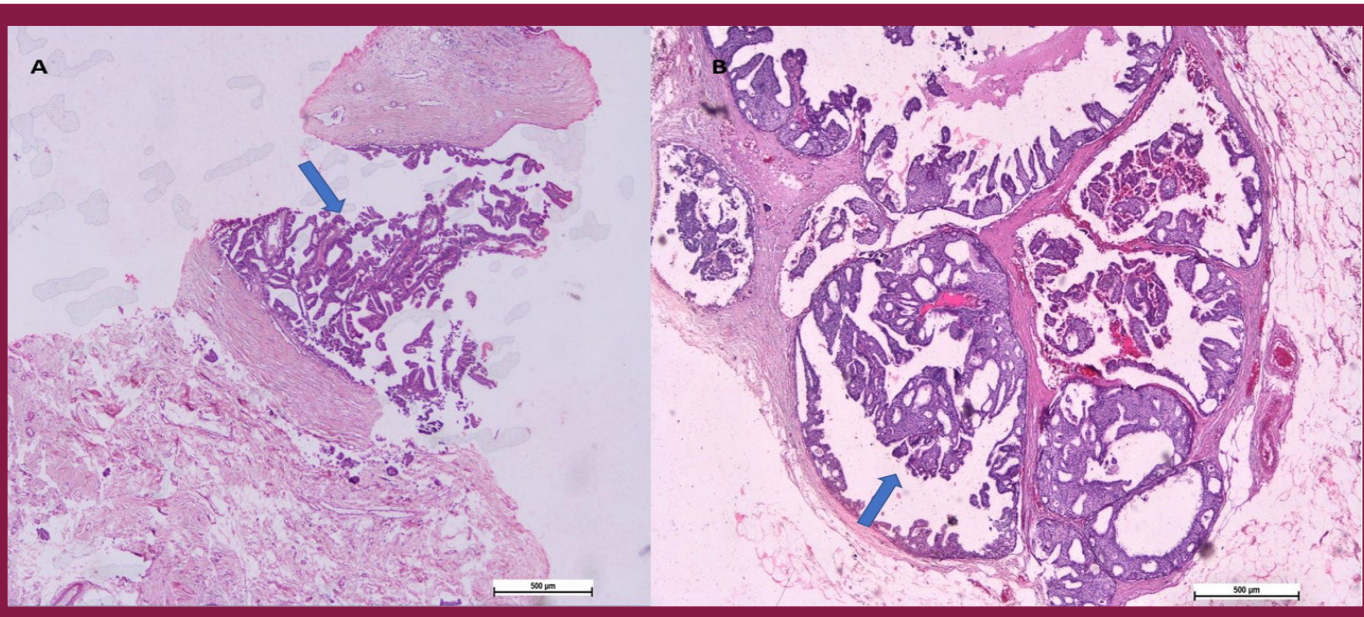

Figure 1. Case 1; a 56-year-old female patient with upgrade. A- Intraductal papilloma diagnosed on core needle biopsy (blue arrow), (H&E, x40) B- Was an upgraded to papillary ductal carcinoma *in situ* on partial mastectomy (blue arrow), (H&E, x40)

In the current study, the upgrade rate for IDP was found to be 5.8%, which was consistent with the literature. There was upgrade to DCIS in 3.5% of patients and to IDC in 2.3%. This risk was greater at older ages, when there were more than 2 papilloma, when a palpable mass was present, and when there was radiological-histopathological discordance.

There is no clear consensus about performing surgical excision after biopsy for FEA. In an analysis of 32 studies, Rudin et al. (21) reported upgrade rates varying between 0% and 42% and calculated the mean value to be 11.1%. Furthermore, detailed evaluation was made of 16 high-quality studies and the upgrade rate was determined to

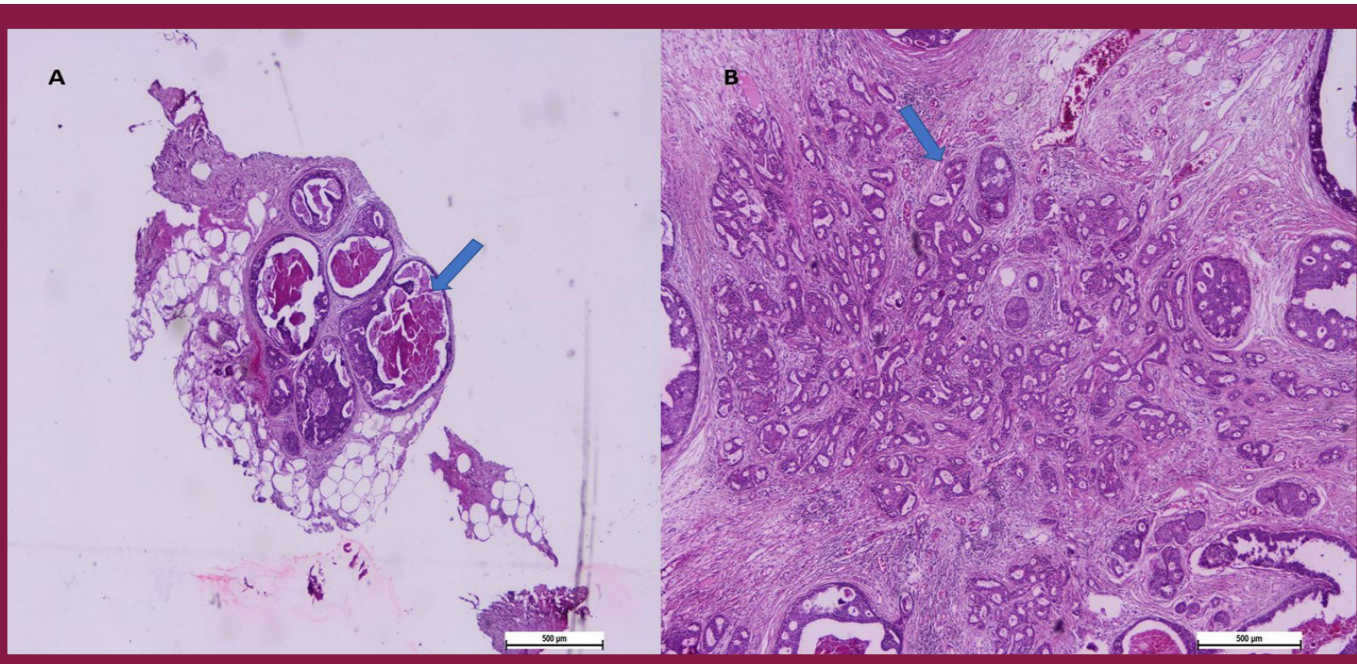

Figure 2. Case 2; 41-year-old female patient with upgrade. A- Ductal carcinoma *in situ* diagnosed on core needle biopsy (blue arrow), (H&E, x40) B- Was an upgraded to invasive ductal carcinoma on partial mastectomy (blue arrow), (H&E, x40)

be 7.5%. In the same study, the upgrade rate to ADH was found to be 18.6% and in conclusion, surgical excision was recommended for FEA (21). Lamb et al. (22) reported the DCIS upgrade risk for FEA as 2.4% and showed that there was ADH, ALH, and LCIS upgrade in 29.8% of patients.

In a similar study, the upgrade rate was reported to be 12%, but the radiological follow-up was recommended as a reasonable option in patients where microcalcifications with characteristics of pure FEA could be completely removed with vacuum-assisted biopsy (23).

In the current study, the upgrade rate for FEA was found to be 16.7%, but there were very few patients diagnosed with FEA in the HRBL group organized in this study.

In the studies in literature related to upgrade rates in ADH, in a meta-analysis by Schiaffino et al. (24) in which 14 studies of the excision of all lesions after biopsy were evaluated, the upgrade rate for IDC was found to be 14% and surgical excision was recommended for patients determined with ADH.

Sutton et al found a similar upgrade rate of 16% for ADH. Of these patients, 81% were upgrade to DCIS and 19% to IDC (25).

Co et al. (26) reported an upgrade rate of 25% and stated that the risk was higher in patients with a mass and suspicious appearance on mammography.

In a study evaluating high-risk lesions, Mooney et al. (27) reported upgrade rates of 18% for patients with ADH and 9% for those with ALH.

Zhao et al. (28) are among the researchers who have found the upgrade risk to be low for ALH, reporting upgrade to DCIS or IDC for only 3.1% of patients. In another study, Cangiarella et al. (29) reported an upgrade rate of 6%.

In the current study, the upgrade rate for ADH was found to be 45.5% and all the patients showed upgrade to IDC. That there was a palpable mass (at least 1 cm) in all the patients with upgrade was found to be worthy of attention.

DCIS is not only high risk but also a precancerous lesion. In a study by Hogue et al. (30), approximately 29.1% of the patients were seen to have upgraded after excision. Lamb et al. (31) found the upgrade risk for DCIS to be 21.8% and reported that the risk increased in those with a family history. Allen et al. (10) determined an upgrade risk of 19.6%.

In the current study, there was seen to be 31.6% upgrade in patients diagnosed with DCIS. These patients were younger than those without upgrade and most had a palpable mass.

Study Limitations

The main limitation of this study was the sample size despite having included all the patients determined with a suspicious mass in the breast and then applied with biopsy. The low number of patients, especially in the FEA and ADH groups, may not have been sufficient to reflect all the results. However, the possibility of encountering upgrade lesion in the excision results of patients diagnosed with HRBL in needle biopsy should be considered.

Take Home Messages

• ADH, ALH, FEA and IDP all indicated an increased risk of *in situ* or invasive breast cancer.

• DCIS indicated an increased risk of invasive breast cancer.

• HRBLs should be evaluated for each patient, along with patient-specific risk factors and imaging findings.

• In patients with HBRL; older ages, the presence of a multifocal lesion, palpable mass, and radiological-histopathological discordance were seen to be risk factors for upgrade.

• Upgrade rates increased especially in IDPs at older ages and in the presence of more than two lesions, and excision should be recommended.

Conclusion

The results of this study showed a general upgrade risk of 10.6% in HRBL, calculated as 5.8% for IDP, 16.7% for FEA, and 45.5% for ADH. This risk was seen to be 31.6% for DCIS. When the patient results were evaluated, older ages, the presence of a multifocal lesion, a palpable mass, and radiological-histopathological discordance were seen to be risk factors for upgrade, especially in the IDP group. A young age and the presence of a palpable mass increase upgrade in DCIS, and in the FEA and ADH groups, the presence of a palpable mass increases the risk.

Ethics

Ethics Committee Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Approval for this study was granted from University of Health Sciences Türkiye, Gaziosmanpaşa Training and Research Hospital Ethics Committee for Clinical Studies in May 2021 (reg: 266).

Informed Consent: Informed consent was not required due to the retrospective use of de-identified administrative data.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.Y., S.B., A.M.E., Concept: E.Y., S.B., N.U., Design: E.Y., Data Collection or Processing: E.Y., M.Ö., A.K., Analysis or Interpretation: E.Y., Literature Search: E.Y., M.Ö., A.K., Writing: E.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Dupont WD, Page DL. Risk factors for breast cancer in women with proliferative breast disease. N Engl J Med. 1985;312:146-151. [Crossref]
2. Bahl M. Management of High-Risk Breast Lesions. Radiol Clin North Am. 2021;59:29-40. [Crossref]
3. Jung SY, Kang HS, Kwon Y, Min SY, Kim EA, Ko KL, et al. Risk factors for malignancy in benign papillomas of the breast on core needle biopsy. World J Surg. 2010;34:261-265. [Crossref]
4. Peres A, Barranger E, Becette V, Boudinet A, Guinebretiere JM, Cherel P. Rates of upgrade to malignancy for 271 cases of flat epithelial atypia (FEA) diagnosed by breast core biopsy. Breast Cancer Res Treat. 2012;133:659-666. [Crossref]
5. Menes TS, Rosenberg R, Balch S, Jaffer S, Kerlikowske K, Miglioretti DL. Upgrade of high-risk breast lesions detected on mammography in the Breast Cancer Surveillance Consortium. Am J Surg. 2014;207:24-31. [Crossref]
6. Page DL, Dupont WD, Rogers LW, Rados MS. Atypical hyperplastic lesions of the female breast. A long-term follow-up study. Cancer. 1985;55:2698-2708. [Crossref]
7. Middleton LP, Sneige N, Coyne R, Shen Y, Dong W, Dempsey P, et al. Most lobular carcinoma in situ and atypical lobular hyperplasia diagnosed on core needle biopsy can be managed clinically with radiologic follow-up in a multidisciplinary setting. Cancer Med. 2014;3:492-499. [Crossref]
8. Virnig BA, Tuttle TM, Shamliyan T, Kane RL. Ductal carcinoma in situ of the breast: a systematic review of incidence, treatment, and outcomes. J Natl Cancer Inst. 2010;102:170-178. [Crossref]
9. Morrow M, Schnitt SJ, Norton L. Current management of lesions associated with an increased risk of breast cancer. Nat Rev Clin Oncol. 2015;12:227-238. [Crossref]
10. Allen A, Cauthen A, Dale P, Jean-Louis C, Lord A, Smith B. Evaluating the frequency of upgrade to malignancy following surgical excision of high-risk breast lesions and ductal carcinoma in situ identified by core needle biopsy. Breast J. 2019;25:103-106. [Crossref]
11. Sickles EA, D'Orsi CJ, Bassett LW, Appleton CM, Berg WA, Burnside ES. ACR BI-RADS mammography, 5th ed. In: D'Orsi CJ, Sickles EA, Mendelson EB, Morris EA, et al. ACR BI-RADS atlas, Breast Imaging Reporting and Data System. Reston, VA: American College of Radiology, 2013. [Crossref]
12. Schnitt SJ, Collins LC. Biopsy Interpretation of the Breast, 3rd edition. Editor: Epstein JI. Lippincott Williams & Wilkins, Wolters Kluwer Health; 2017:62-112. [Crossref]
13. Said SM, Visscher DW, Nassar A, Frank RD, Vierkant RA, Frost MH, et al. Flat Epithelial Atypia and Risk of Breast Cancer: A Mayo Cohort Study. Cancer. 2015;121:1548-1555. [Crossref]
14. Myers DJ, Walls AL. Atypical Breast Hyperplasia. In: StatPearls (Internet). Treasure Island (FL): StatPearls Publishing; 2021. [Crossref]
15. Lester SC, Bose S, Chen YY, Connoly JL, de Baca ME, Fitzgibbons PL, et al. Protocol for the examination of specimens from patients with ductal carcinoma in situ of the breast. Arch Pathol Lab Med. 2009;133:15-25. [Crossref]
16. Brennan ME, Turner RM, Ciatto S, Marinovich ML, French JR, Macaskill P, et al. Ductal carcinoma in situ at core-needle biopsy: meta-analysis of underestimation and predictors of invasive breast cancer. Radiology. 2011;260:119-128. [Crossref]
17. Lamb LR, Lehman CD, Oseni TO, Bahl M. Ductal Carcinoma In Situ (DCIS) at Breast MRI: Predictors of Upgrade to Invasive Carcinoma. Acad Radiol. 2020;27:1394-1399. [Crossref]

18. MacCol C, Salehi A, Parpia S, Hodgson N, Ramonas M, Williams P. Benign breast papillary lesions diagnosed on core biopsy: upgrade rate and risk factors associated with malignancy on surgical excision. Virchows Arch. 2019;475:701-707. [Crossref]
19. Qui L, Mais DD, Nicolas M, Nanyes J, Kist K, Nazarullah A. Diagnosis of Papillary Breast Lesions on Core Needle Biopsy: Upgrade Rates and Interobserver Variability. Int J Surg Pathol. 2019;27:736-743. [Crossref]
20. Han SH, Kim M, Chung YR, Yun B, Jang M, Kim SM, et al. Benign Intraductal Papilloma without Atypia on Core Needle Biopsy Has a Low Rate of Upgrading to Malignancy after Excision. J Breast Cancer. 2018;21:80-86. [Crossref]
21. Rudin AV, Hoskin TL, Fahy A, Farrell AM, Nassar A, Ghosh K, et al. Flat Epithelial Atypia on Core Biopsy and Upgrade to Cancer: a Systematic Review and Meta-Analysis. Ann Surg Oncol. 2017;24:3549-3558. [Crossref]
22. Lamb LR, Bahl M, Gadd MA, Lehman CD. Flat Epithelial Atypia: Upgrade Rates and Risk-Stratification Approach to Support Informed Decision Making. J Am Coll Surg. 2017;225:696-701. [Crossref]
23. DisPasquale A, Silverman S, Farag E, Peiris L. Flat epithelial atypia: are we being too aggressive? Breast Cancer Res Treat. 2020;179:511-517. [Crossref]
24. Schiaffino S, Calabrese M, Melani EF, Trimboli RM, Cozzi A, Carbonaro LA, et al. Upgrade Rate of Percutaneously Diagnosed Pure Atypical Ductal Hyperplasia: Systematic Review and Meta-Analysis of 6458 Lesions. Radiology. 2020;294:76-86. [Crossref]
25. Sutton T, Farinola M, Johnson N, Garreau JR. Atypical ductal hyperplasia: Clinicopathologic factors are not predictive of upgrade after excisional biopsy. Am J Surg. 2019;217:848-850. [Crossref]
26. Co M, Kwong A, Shek T. Factors affecting the under-diagnosis of atypical ductal hyperplasia diagnosed by core needle biopsies - A 10-year retrospective study and review of the literature. Int J Surg. 2018;49:27-31. [Crossref]
27. Mooney KL, Bassett LW, Apple SK. Upgrade rates of high-risk breast lesions diagnosed on core needle biopsy: a single-institution experience and literature review. Mod Pathol. 2016;29:1471-1484. [Crossref]
28. Zhao C, Desouki MM, Florea A, Mohammed K, Li X, Dabbs D. Pathologic findings of follow-up surgical excision for lobular neoplasia on breast core biopsy performed for calcification. Am J Clin Pathol. 2012;138:72-78. [Crossref]
29. Cangiarella J, Guth A, Axelrod D, Darvishian F, Singh B, Simsir A, et al. Is surgical excision necessary for the management of atypical lobular hyperplasia and lobular carcinoma in situ diagnosed on core needle biopsy?: A report of 38 cases and review of the literature. Arch Pathol Lab Med. 2008;132:979-983. [Crossref]
30. Hogue JC, Morais L, Provencher L, Desbiens C, Poirier B, Poirier É, et al. Characteristics associated with upgrading to invasiveness after surgery of a DCIS diagnosed using percutaneous biopsy. Anticancer Res. 2014;34:1183-1191. [Crossref]
31. Lamb LR, Kim G, Oseni TO, Bahl M. Noncalcified Ductal Carcinoma in Situ (DCIS): Rate and Predictors of Upgrade to Invasive Carcinoma. Acad Radiol. 2021;28:e71-e76. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2022.49469

Hamidiye Med J 2022;3(2):118-123

The Relationship Between Vitamin D Level and Prognostic Factors in Patients Diagnosed with Breast Cancer

Meme Kanseri Tanılı Hastalarda D Vitamini Düzeyi ile Prognostik Faktörler Arasındaki İlişki

Levent Emirzeoğlu[1], Serdar Arıcı[2]

[1]*University of Health Sciences Türkiye, İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital, Clinic of Medical Oncology, İstanbul, Türkiye*

[2]*University of Health Sciences Türkiye, Prof. Dr. Cemil Taşçıoğlu City Hospital, Clinic of Medical Oncology, İstanbul, Türkiye*

ABSTRACT

Background: To examine the relationship between vitamin D level at the time of diagnosis and disease prognostic factors in patients diagnosed with breast cancer.

Materials and Methods: We studied 150 patients with breast cancer whose vitamin D levels were measured before treatment. The patients' vitamin D levels were compared with their estrogen receptor (ER) levels, progesterone receptor (PR) levels, human epidermal growth factor receptor 2 (Her-2/neu) status, ki-67 levels, and histopathological features.

Results: Vitamin D was found to be significantly positively correlated with ER (p<0.001) and PR (p=0.032) staining intensities and significantly negatively correlated with ki-67 level (p=0.001). Both the ER (p=0.003) and ki-67 index (p=0.024) were found to be significantly correlated with vitamin D level, 20 ng/mL below and above, and its relation with prognostic factors.

Conclusion: There may be a relationship between serum 25 hydroxy vitamin D [25(OH)D] level and breast cancer prognosis. Our study can be used as a guide for examining the contribution of supportive treatments that provide normal vitamin D levels (>30 ng/mL) to decrease breast cancer aggressiveness. More studies are needed to elucidate the relationship between breast cancer and vitamin D.

Keywords: Vitamin D, breast cancer, prognosis

ÖZ

Amaç: Meme kanseri tanılı hastalarda tanı anındaki D vitamini düzeyi ile hastalığın prognostik faktörleri arasındaki ilişkiyi incelemektir.

Gereç ve Yöntemler: Tedavi öncesi D vitamini düzeyi ölçülen 150 meme kanserli hastayı inceledik. Hastaların D vitamini düzeyleri östrojen reseptör (ER) düzeyleri, progesteron reseptör (PR) düzeyleri, insan epidermal büyüme faktörü reseptörü 2 (Her-2/neu) durumu, ki-67 düzeyleri ve histopatolojik özellikleri ile karşılaştırıldı.

Bulgular: D vitamininin ER (p<0,001) ve PR (p=0,032) boyama yoğunlukları ile anlamlı derecede pozitif, ki-67 düzeyi (p=0,001) ile anlamlı derecede negatif korelasyon gösterdiği bulundu. Hem ER (p=0,003) hem de ki-67 indeksi (p=0,024), 20 ng/mL altı ve üstü D vitamini düzeyleri ile anlamlı olarak ilişkili bulundu.

Sonuç: Serum 25 hidroksi vitamin D [25(OH)D] düzeyi ile meme kanseri prognozu arasında bir ilişki olabilir. Çalışmamız, normal D vitamini düzeylerini (>30 ng/mL) sağlayan destekleyici tedavilerin meme kanseri agresifliğini azaltmaya katkısını incelemek için bir rehber olarak kullanılabilir. Meme kanseri ve D vitamini arasındaki ilişkiyi aydınlatmak için daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: D vitamini, meme kanseri, prognoz

Address for Correspondence: Levent Emirzeoğlu, University of Health Sciences Türkiye, İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital, Clinic of Medical Oncology, İstanbul, Türkiye

Phone: +90 216 542 20 20 E-mail: emirzeoglulevent@hotmail.com **ORCID ID:** orcid.org/0000-0002-7905-986X

Received: 21.03.2022 **Accepted:** 15.06.2022

©Copyright 2022 by University of Health Sciences Türkiye Hamidiye Faculty of Medicine. / Hamidiye Medical Journal published by Galenos Yayınevi.

Introduction

Globally, breast cancer is the most frequently diagnosed malignancy. It is also the leading cause of cancer death in women worldwide (1). Accumulated evidence indicates that various genetic and environmental factors may be associated with the initiation and progression of breast cancer. Ecological studies have found an inverse relationship between sunlight exposure and breast cancer risk (2).

Some factors have been shown to affect the course of the disease in breast cancer patients. These include histopathological features (type, grade, tumor stage, lymph node status), ki-67 level, estrogen receptor (ER) status, progesterone receptor (PR) status, and human epidermal growth factor receptor 2 (Her-2) status. These factors can be affected by both genetics and socioeconomic status (3,4). Tumors positive for ER and PR have a better prognosis (5). Her-2 overexpression indicates a poor prognosis, especially if patients are not treated with chemotherapy and HER-2-directed agents (6). Diagnosis under 35 years of age, presenting with locally advanced disease, tumor size ⩾2 cm, axillary lymph node involvement, and high histological grade are poor prognostic criteria (7,8,9,10).

Vitamin D is a sterol derivative, and 25 hydroxy vitamin D [25(OH)D] is its main circulating form (11). Measurement of serum 25(OH)D concentration is the best laboratory test for vitamin D level. The lower limit of normal for 25(OH)D levels varies according to geographic location and the average amount of time the population is exposed to sunlight. According to the United States Medical Association, a serum 25(OH)D concentration of 20 ng/mL (50 nmol/L) and above is generally considered sufficient, while in other guidelines, the minimum amount required to minimize the risk of falls and fractures is 30 ng/mL (75 nmol/L) (12).

Numerous epidemiological studies have investigated the relationship between serum 25(OH)D level and breast cancer risk. In a review including nine prospective studies with 11,656 women in the postmenopausal period, it was shown that every 5 ng/mL increase in serum 25(OH)D level reduced the risk of breast cancer by 12% (13). In contrast, a randomized study conducted with 36,282 postmenopausal women found no relationship between serum 25(OH)D level and breast cancer risk when the group receiving 1.000 mg of elemental calcium and 400 units of vitamin D3 daily was compared with the placebo group (14). In a meta-analysis, vitamin D was shown to positively affect breast cancer survival (15). In another meta-analysis, newly diagnosed breast cancer patients had significantly lower serum 25(OH)D levels than healthy controls. A lower level of serum 25(OH)D has been correlated with aggressive breast cancer phenotypes (16).

Material and Methods

Study Population

Our study was carried out with patients who applied to University of Health Sciences Türkiye (Okmeydanı Training and Research Hospital and İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital Oncology Outpatient Clinic. Our study protocol complied with the principles of the Declaration of Helsinki and was approved by the University of Health Sciences Türkiye, Prof. Dr. Cemil Taşçıoğlu Hospital Clinical Research Ethics Committee (05/11/2019, number: 1471). The files of 624 patients with newly diagnosed premenopausal and postmenopausal breast cancer between January 2018 and August 2021 were scanned. We excluded 286 patients due to a lack of vitamin D value, 64 patients who were perimenopausal, and 124 patients due to distant organ metastasis. Thus, 150 female cases were included in the study. The case information was examined, and 25(OH)D levels at the time of diagnosis were recorded. The relationship between ER, PR, Her-2/neu, histological diagnosis, grade, tumor diameter, ki-67 status, lymph node metastasis status, and 25(OH)D levels were evaluated statistically. The values measured for 25(OH)D before and after the diagnosis during the 20-day period without antitumoral treatment were noted.

Statistical Analysis

Statistical analyses were performed using SPSS v. 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were given as a number and as a percentage for categorical variables, average, standard deviation, and minimum and maximum for numeric variables. The numerical variables did not meet the normal distribution condition, and comparisons of more than two independent groups were made using the Kruskal-Wallis test and comparisons of two independent groups were made using the Mann-Whitney U test. Comparisons of the ratios in the groups were made using the chi-square test. Spearman's correlation test was used to determine the level of correlation between vitamin D and prognostic factors. Statistical significance level of alpha was accepted as $p<0.05$.

Results

A total of 150 newly diagnosed female patients with breast cancer were included in the study. The mean age of the patients was 47.9±11.2 (range: 22-81) years. Of these, 85 patients (56.7%) were premenopausal, and 65 (43.3%) were postmenopausal. There were 48 (32.0%) ER-negative and 102 (68.0%) ER-positive patients. ER +, ++, and +++

patient numbers were 33 (22.0%), 65 (43.3%), and 4 (2.7%), respectively. The number of PR negative patients was 74 (49.3%), while the number of PR positive patients was 76 (50.7%). The number of PR +, ++, and +++ patients was 35 (23.3%), 32 (21.3%), and 9 (6.0%), respectively. The numbers of Her-2-negative and -positive patients were 113 (75.3%) and 37 (24.7%), respectively. All patients had invasive ductal cancer histology. When histological grades were examined, 8 patients (5.3%) were grade 1, 100 (66.7%) were grade 2, and 42 (28.0%) were grade 3. The mean tumor diameter of the patients was calculated as 23.2±16.3 mm (range: 1-85 mm). The mean ki-67 levels were 35.7%±25.4% (range: 2-90). The mean 25(OH)D levels were 17.8±10.2 ng/mL (range: 3.8-79.5 ng/mL) (Table 1).

In the correlation analysis between 25(OH)D levels and clinicopathological data, no correlation was found with age, Her-2 status, tumor diameter, or tumor grade. A statistically significant positive correlation was found for vitamin D with ER (p=0.306, p<0.001) and PR (p=0.175, p=0.032) staining intensities. A statistically significant negative correlation was observed between 25(OH)D levels and ki-67 (p=0.300, p=0.001) (Table 2).

When the relationship of patients' 25(OH)D levels was compared with ER, PR, Her-2, and menopause status, the mean vitamin D levels of ER-negative and -positive patients were 15.0±11.2 ng/mL and 19.1±9.5 ng/mL, respectively. For ER-positive patients, 25(OH)D levels were significantly higher than ER-negative patients (p=0.002). The mean vitamin D levels of PR-negative and -positive patients were 17.3±11.9 ng/mL and 18.2±8.2 ng/mL, respectively. Although 25(OH)D levels were lower for PR-negative patients than PR-positive patients, this difference was not significant. When analyzed according to menopause status and Her-2 status, no statistically significant differences were found between the groups (Table 3).

The patients' ER, PR, ki-67 status, Her2-neu status, menopausal status, lymph node metastasis, tumor grade, and tumor diameter were examined according to vitamin D levels. A statistically significant correlation was found between vitamin D level and ER (p=0.003). The ki-67 level was divided into two groups: Below and above 14%. There was a statistically significant relationship between vitamin D and ki-67 status (p=0.024) (Table 4).

Discussion

Breast cancer is the most common malignancy among women worldwide (17). Breast cancer subtypes were classified according to the expression of ER, PR, and HER2. The prognosis of each subtype was also closely associated with the expression of those receptors (18). Breast cancer cases with ER+ subtypes are associated with the best prognosis. By contrast, women with ER- subtypes, especially those with

Table 1. The patient's clinicopathological features

Age			**47.9±11.2 (22-81)**
ER	Negative (n=48)	Negative	48 (32.0%)
		+	33 (22.0%)
	Positive (n=102)	++	65 (43.3%)
		+++	4 (2.7%)
PR	Negative (n=74)	Negative	74 (49.3%)
		+	35 (23.3%)
	Positive (n=76)	++	32 (21.3%)
		+++	9 (6.0%)
Her-2		Negative	113 (75.3%)
		Positive	37 (24.7%)
Menopause		Postmenopause	65 (43.3%)
		Premenopause	85 (56.7%)
Tumor diameter (mm)			23.2±16.3 (1-85)
Grade		1	8 (5.3%)
		2	100 (66.7%)
		3	42 (28.0%)
Ki-67%			35.7±25.4 (2-90)
Vitamin D level (ng/mL)			17.8±10.2 (3.8-79.5)

ER: Estrogen receptor, PR: Progesterone receptor, Her-2: Human epidermal growth factor receptor-2

triple-negative disease, suffer the worst prognosis (19). Multiple studies have shown associations between adequate circulating 25(OH)D levels, and decreased prognosis of breast cancer (20,21). In recent meta-analysis of 12 cohort studies involving 8.574 breast cancer patients suggested that low 25-hydroxyvitamin D level was associated with a worse survival (22). In breast cancer tissues, vitamin D has anticancer effects that are mediated through vitamin D reseptor acting as a transcription factor and regulating several genes with an antiproliferative, proapoptotic, and differentiation action (23). Vitamin D induces ER expression in ER-negative breast cancers, thereby restoring their response to anti-estrogens (24).

We investigated the relationship between serum 25(OH) D levels at the time of diagnosis and disease prognostic factors in 150 premenopausal and postmenopausal women with breast cancer. In a study conducted in 192 postmenopausal breast cancer patients, the relationship between 25(OH)D levels at the time of diagnosis and prognostic data was examined. Individuals with low 25(OH) D levels were found to be more likely to have positive lymph nodes, lower ER and PR ratios, and higher ki-67 levels (25).

Table 2. The correlation between vitamin D and prognostic factors

	Vitamin D level	
	rho	**p**
Age	0.024	0.773
ER	0.306	**<0.001**
PR	0.175	**0.032**
Her-2	-0.071	0.387
Ki-67	-0.300	**0.001**
Tumor diameter	-0.017	0.851
Grade	-0.159	0.070

ER: Estrogen receptor, PR: Progesterone receptor, Her-2: Human epidermal growth factor receptor-2

In our study, we found that patients with low serum 25(OH) D levels had significantly lower ER and PR rates and higher ki-67 levels compared to those with high serum 25(OH)D levels. However, we did not find such a relationship with lymph node status.

In a study, serum 25(OH)D levels at the time of diagnosis were investigated from 50 female patients without primary invasive metastatic disease, and as a result, a significantly larger tumor size was observed in patients with low serum vitamin D levels (26). In a similar study, the 25-hydroxyvitamin D level had a significant inverse association with metastatic breast cancer. Low vitamin D levels were associated with advanced stages of the disease, tumor size, and grade in postmenopausal patients (27). However, in our study, we divided the serum 25(OH)D level into two groups (< and >20 ng/mL) and found no significant correlation between tumor size and serum 25(OH)D level.

Abdel-Rezaq (28) showed that patients with low vitamin D levels had larger tumor sizes (2.9% vs. 46.7%), more advanced disease (2.9% vs. 53.3%), higher grade tumors (33.3% vs. 2.9%), negative hormone receptors (73.3% vs. 51.4%), and higher Her-2 positive values (40.0% vs. 86.7%). Similarly, in our study, a positive correlation was found for vitamin D level with ER and PR staining intensities. However, no significant correlation was found with tumor diameter, grade, or Her-2 positivity. The 150 patients included in our study were both post-menopausal and pre-menopausal patients.

Peppone et al. (29) compared the 25(OH)D levels of 194 women who had undergone breast cancer surgery and 194 cancer-free controls. Vitamin D levels of breast cancer cases were found to be significantly lower than the control group. Women with serum 25(OH)D levels below 32 ng/mL had a significantly higher rate of ER negativity and triple negative cancer. In our study, 25(OH)D levels of ER-positive patients were found to be statistically significantly higher than in negative patients.

Table 3. Vitamin D levels in prognostic groups

		Vitamin D level (ng/mL)		
		Mean ± SD	**Min-max (median)**	**p**
ER	Negative	15.0±11.2	3.8-79.5 (13.7)	**0.002**
	Positive	19.1±9.5	3.9-52.9 (18.3)	
PR	Negative	17.3±11.9	3.8-79.5 (15.9)	0.073
	Positive	18.2±8.2	3.9-43.8 (18.6)	
Her-2	Negative	18.3±10.8	3.8-79.5 (17.0)	0.445
	Positive	16.8±9.1	3.9-52.9 (14.1)	
Menopause	Postmenopause	17.2±11.2	3.9-79.5 (16.3)	0.841
	Premenopause	18.2±9.4	3.8-52.9 (17.3)	

SD: Standard deviation, ER: Estrogen receptor, PR: Progesterone receptor, Her-2: Human epidermal growth factor receptor-2

Table 4. The relationship of vitamin D levels with prognostic factors

Variable		D vit<20 ng/mL	D vit>20 ng/mL	*p
ER n=150	Negative	41	7	**p=0.003**
	Positive	63	39	
PR n=150	Negative	50	18	p=0.096
	Positive	48	28	
Ki-67 n=121	<14%	10	12	**p=0.024**
	>14%	70	29	
Menopause n=150	Premenopause	48	17	p=0.295
	Postmenopause	56	29	
Her-2 n=150	Negative	63	31	p=0.426
	Positive	41	15	
Lymph node metastasis n=75	None	22	8	p=0.169
	Detected	26	19	
Grade n=131	Low	62	34	p=0.173
	High	27	8	
Tumor diameter n=121	<20 mm	41	17	p=0.634
	>20 mm	42	21	

*Chi-square test, ER: Estrogen receptor, PR: Progesterone receptor, Her-2: Human epidermal growth factor receptor-2

Study Limitations

The relationship between cancer and vitamin D has been investigated in many studies. We examined the relationship between vitamin D and disease prognostic factors. The limitation of this study is that it was retrospective.

Conclusion

Low vitamin D levels at the time of diagnosis in breast cancer are associated with low ER and PR levels and a high ki-67 index, which have been proven to be prognostic factors for breast cancer. These findings show that adequate vitamin D level can affect breast cancer prognosis in a good way.

Ethics

Ethics Committee Approval: Our study was carried out with patients who applied to University of Health Sciences Türkiye, (Prof. Dr. Cemil Taşçıoğlu Hospital and İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital Oncology Outpatient Clinic. Our study protocol complied with the principles of the Declaration of Helsinki and was approved by the University of Health Sciences Türkiye, Prof. Dr. Cemil Taşçıoğlu Hospital Clinical Research Ethics Committee (date: 05/11/2019, number: 1471).

Informed Consent: Retrospective study.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Concept: L.E., S.A., Design: L.E., S.A., Data Collection or Processing: L.E., S.A., Analysis or Interpretation: L.E., S.A., Literature Search: S.A., Writing: L.E.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424. [Crossref]
2. Gnagnarella P, Raimondi S, Aristarco V, Johansson H, Bellerba F, Corso F, et al. Ethnicity as modifier of risk for Vitamin D receptors polymorphisms: Comprehensive meta-analysis of all cancer sites. Crit Rev Oncol Hematol. 2021;158:103202. [Crossref]
3. Nicolini A, Ferrari P, Duffy MJ. Prognostic and predictive biomarkers in breast cancer: Past, present and future. Semin Cancer Biol. 2018;52:56-73. [Crossref]
4. Tarighati E, Keivan H, Mahani H. A review of prognostic and predictive biomarkers in breast cancer. Clin Exp Med. 2022. [Crossref]
5. Hoda SA, Rosen PP, Brogi E, Koerner FC. Rosen's breast pathology. Lippincott Williams & Wilkins; 2020. [Crossref]
6. Loibl S, Gianni L. HER2-positive breast cancer. Lancet. 2017;389:2415-2429. [Crossref]
7. Fredholm H, Eaker S, Frisell J, Holmberg L, Fredriksson I, Lindman H. Breast cancer in young women: poor survival despite intensive treatment. PloS one. 2009;4:e7695. [Crossref]

8. Bastiaannet E, Liefers GJ, de Craen AJ, Kuppen PJ, Van De Water W, Portielje JE, et al. Breast cancer in elderly compared to younger patients in the Netherlands: stage at diagnosis, treatment and survival in 127,805 unselected patients. Breast Cancer Res Treat. 2010;124:801-807. [Crossref]
9. Newman LA. Epidemiology of locally advanced breast cancer. Semin Radiat Oncol. 2009;19:195-203. [Crossref]
10. Galea MH, Blamey RW, Elston CE, Ellis IO. The Nottingham Prognostic Index in primary breast cancer. Breast cancer res treat. 1992;22:207-219. [Crossref]
11. Lips P. Vitamin D physiology. Prog biophys mol biol. 2006;92:4-8. [Crossref]
12. Dawson-Hughes B, Mithal A, Bonjour JP, Boonen S, Burckhardt P, Fuleihan GE, et al. IOF position statement: vitamin D recommendations for older adults. Osteoporosis Int. 2010;21:1151-1154. [Crossref]
13. Bauer SR, Hankinson SE, Bertone-Johnson ER, Ding EL. Plasma vitamin D levels, menopause, and risk of breast cancer: dose-response meta-analysis of prospective studies. Medicine (Baltimore). 2013;92:123-131. [Crossref]
14. Chlebowski RT, Wactawski-Wende J, Ritenbaugh C, Hubbell FA, Ascensao J, Rodabough RJ, et al. Estrogen plus progestin and colorectal cancer in postmenopausal women. N Engl J Med. 2004;350:991-1004. [Crossref]
15. Kim Y, Je Y. Vitamin D intake, blood 25 (OH) D levels, and breast cancer risk or mortality: a meta-analysis. Br J Cancer. 2014;110:2772-2784. [Crossref]
16. Karthikayan A, Sureshkumar S, Kadambari D, Vijayakumar C. Low serum 25-hydroxy vitamin D levels are associated with aggressive breast cancer variants and poor prognostic factors in patients with breast carcinoma. Arch Endocrinol Metab. 2018;62:452-459. [Crossref]
17. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71:209-249. [Crossref]
18. Hwang KT, Kim J, Jung J, Chang JH, Chai YJ, Oh SW, et al. Impact of breast cancer subtypes on prognosis of women with operable invasive breast cancer: a population-based study using SEER database. Clin Cancer Res. 2019;25:1970-1979. [Crossref]
19. Howlader N, Cronin KA, Kurian AW, Andridge R. Differences in breast cancer survival by molecular subtypes in the United States. Cancer epidemiol biomarkers prev. 2018;27:619-626. [Crossref]
20. 20. Hu K, Callen DF, Li J, Zheng H. Circulating vitamin D and overall survival in breast cancer patients: a dose-response meta-analysis of cohort studies. Integr Cancer Ther. 2018;17:217-225. [Crossref]
21. Hossain S, Beydoun MA, Beydoun HA, Chen X, Zonderman AB, Wood RJ. Vitamin D and breast cancer: A systematic review and meta-analysis of observational studies. Clin Nutr ESPEN. 2019;30:170-184. [Crossref]
22. Li C, Li H, Zhong H, Li X. Association of 25-hydroxyvitamin D level with survival outcomes in female breast cancer patients: A meta-analysis. J Steroid Biochem Mol Biol. 2021;212:105947. [Crossref]
23. Zhang X, Harbeck N, Jeschke U, Doisneau-Sixou S. Influence of vitamin D signaling on hormone receptor status and HER2 expression in breast cancer. J Cancer Res Clin Oncol. 2017;143:1107-1122. [Crossref]
24. Segovia-Mendoza M, García-Quiroz J, Díaz L, García-Becerra R. Combinations of Calcitriol with Anticancer Treatments for Breast Cancer: An Update. Int J Mol Sci. 2021;22:12741. [Crossref]
25. de Sousa Almeida-Filho B, De Luca Vespoli H, Pessoa EC, Machado M, Nahas-Neto J, Nahas EAP. Vitamin D deficiency is associated with poor breast cancer prognostic features in postmenopausal women. J Steroid Biochem Mol Biol. 2017;174:284-289. [Crossref]
26. Ismail A, El-Awady R, Mohamed G, Hussein M, Ramadan SS. Prognostic significance of serum vitamin D levels in Egyptian females with breast cancer. Asian Pacific J cancer prev. 2018;19:571-576. [Crossref]
27. Janbabai G, Shekarriz R, Hassanzadeh H, Aarabi M, Borhani SS. A survey on the relationship between serum 25-hydroxy vitamin D level and tumor characteristics in patients with breast cancer. Int J Hematol Oncol Stem Cell Res. 2016;10:30-36. [Crossref]
28. Abdel-Razeq H. Prognostic Significance of Serum Vitamin D Levels in Egyptian Females with Breast Cancer. Asian Pac J Cancer Prev. 2019;20:983-983. [Crossref]
29. Peppone LJ, Rickles AS, Janelsins MC, Insalaco MR, Skinner KA. The association between breast cancer prognostic indicators and serum 25-OH vitamin D levels. Ann Surg Oncol. 2012;19:2590-2599. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2022.03164

Hamidiye Med J 2022;3(2):124-131

Can the Progression of COVID-19 Pneumonia be Predicted?

COVID-19 Pnömonisinin Progresyonu Öngörülebilir mi?

Fatma Demirci Üçsular[1], Gülistan Karadeniz[1], Gülru Polat[1], Damla Serçe Unat[1], Aysu Ayrancı[2], Enver Yalnız[1], Filiz Güldaval[1]

[1]*University of Health Sciences Türkiye, Dr. Suat Seren Chest Disease and Surgery Training and Research Hospital, Clinic of Chest Diseases, İzmir, Türkiye*

[2]*Bakırçay University Çiğli Training and Research Hospital, Department of Chest Diseases, İzmir, Türkiye*

ABSTRACT

Background: Coronavirus disease-2019 (COVID-19) remains a major cause of morbidity and mortality. There are many parameters affecting the progression of the disease. The purpose of the present study was to evaluate and compare the initial data of patients hospitalized with the diagnosis of COVID-19 pneumonia, who progressed during the hospitalization period, with other patients who recovered or remained stable, and to investigate the risk factors that can be used to predict the disease progression.

Materials and Methods: Patients, who received inpatient treatment with the diagnosis of COVID-19 pneumonia, were included in the study retrospectively. Two groups were created from all patients according to their progression in hospital follow-ups: Group 1: Progression group and group 2: Recovery/stabilization group. If patients had clinical, laboratory and/or radiological deterioration or died during follow-up, these patients were included in the progression group. If patients recovered or remained stable, these patients were also included in the recovery/stabilization group. The demographic data, initial hemogram, biochemical parameters and radiological data of the patients were recorded.

Results: It was determined in the univariate analysis that the age, smoking status, comorbidity, heart disease, chronic obstructive pulmonary disease, cancer, dyspnea, fever, leukocytosis, lymphopenia, elevated neutrophil-lymphocyte ratio (NLR), C-reactive protein, albumin, lactate dehydrogenase, ferritin, D-dimer, troponin-T, pro-B-type natriuretic peptide (pro-BNP) were risk factors predicting disease progression all p-values<0.05. In the multivariate logistic regression analysis, it was found that fever, NLR, and D-dimer could be used to predict the disease progression (p<0.05). In the ROC analysis, the sensitivity of NLR was 83.3%, specificity 57.5%, and cut-off >3.545 [area under curve (AUC)=0.752; p<0.001]; the sensitivity of pro-BNP was 71.8%, specificity 73.8%, and cut-off >332.8 (AUC=0.752; p<0.001), the sensitivity of troponin-T was 81.2%, specificity was 60.6%, and cut-off was >4.58 (AUC=0.730; p<0.001) in predicting progression.

Conclusion: The identification of risk factors predicting progression is important in reducing morbidity and mortality rates. Fever, NLR, D-dimer troponin-T and pro-BNP are important parameters that can be used to predict progression.

Keywords: COVID-19 pneumonia, progression, risk factors

ÖZ

Amaç: Koronavirüs hastalığı-2019 (COVID-19) önemli bir morbidite ve mortalite nedeni olmaya devam etmektedir. Hastalığın ilerlemesini etkileyen birçok parametre vardır. Çalışmanın amacı; COVID-19 pnömonisi tanısı ile hastaneye yatırılan ve yatış süresi boyunca progresyon gösteren hastaların ilk verilerini, iyileşen veya stabil kalan diğer hastalarla karşılaştırmak ve progresyonu öngörmede kullanılabilecek risk faktörlerini araştırmaktır.

Gereç ve Yöntemler: COVID-19 pnömonisi tanısıyla yatarak tedavi alan hastalar retrospektif olarak çalışmaya dahil edildi. Tüm hastalardan hastane takiplerindeki seyirlerine göre 2 grup oluşturuldu: 1. grup: Progresyon grubu ve 2. grup: İyileşme/stabilizasyon grubu. Hastalarda klinik, laboratuvar ve/veya radyolojik kötüleşme görüldüyse veya takipler sırasında hastalar eks olduysa, bu hastalar progresyon grubuna alındı. İyileşme veya stabil seyrettiyse, bu hastalar da iyileşme/stabilizasyon grubuna dahil edildi. Hastaların demografik verileri, başlangıç hemogram ve biyokimyasal parametreleri ve radyolojik verileri kaydedildi.

Bulgular: Univariate analizde; yaş, sigara, komorbidite, kalp hastalığı, KOAH, kanser, nefes darlığı, ateş, lökositoz, lenfopeni, nötrofil-lenfosit oranı (NLR) yüksekliği, C-reaktif protein, albümin, laktat dehidrogenaz, ferritin, D-dimer, troponin T, pro-B tipi natriüretik

Address for Correspondence: Fatma Demirci Üçsular, University of Health Sciences Türkiye, Dr. Suat Seren Chest Disease and Surgery Training and Research Hospital, Clinic of Chest Diseases, İzmir, Türkiye

Phone: +90 544 645 27 67 E-mail: fatmaucsular@gmail.com **ORCID ID:** orcid.org/0000-0003-3746-3095

Received: 21.04.2022 **Accepted:** 14.06.2022

©Copyright 2022 by University of Health Sciences Türkiye Hamidiye Faculty of Medicine. / Hamidiye Medical Journal published by Galenos Yayınevi.

ÖZ

peptitin (pro-BNP) hastalığın progresyonunu öngören risk faktörleri olduğunu saptadık (tüm p-değerleri<0,05). Multivariate logistic regression analizinde; sıcaklık, NLR ve D-dimerin progresyonu predikte etmede kullanılabileceğini saptadık (p<0,05). ROC analizinde; progresyonu öngörmede; NLR'nin sensivitesi %83,3, spesifitesi %57,5, cut-off >3,545 [eğri altındaki alan (EAA)=0,752; p<0,001], pro-BNP'nin sensivitesi %71,8, spesifitesi %73,8, cut-off >332,8 (EAA=0,752; p<0,001), troponin-T'nin sensivitesi %81,2, spesifitesi %60,6, cut-off >4.58 (EAA=0,730; p<0,001) olarak belirledik.

Sonuç: Progresyonu öngören risk faktörlerinin belirlenmesi morbidite ve mortalite oranlarını azaltmada önemlidir. Ateş, NLR, D-dimer troponin-T ve pro-BNP progresyonu öngörmede kullanılabilecek önemli parametrelerdir.

Anahtar Kelimeler: COVID-19 pnömonisi, progresyon, risk faktörleri

Introduction

Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) has a highly contagiouse and pathogenicity and has caused "Coronavirus disease-2019 (COVID-19)" since the last months of 2019 and the pandemic process continues.

The clinical manifestations of COVID-19 appear in a wide range from asymptomatic to critical disease and mortality (1). Because of the overcrowding of hospitals due to COVID-19 pandemic, studies were needed on which parameters could be used to predict the worsening of the disease and mortality in terms of demographic, clinical, hematological, biochemical, and radiological data. In the present study, the purpose was to compare the initial data of the patients who were hospitalized with the diagnosis of COVID-19 and who had progression during the hospitalization period with other patients who recovered or remained stable and to investigate the risk factors that can be used to predict the disease progression.

Material and Methods

This study was conducted following the Declaration of Helsinki and approved by the Ethics Committee of University of Health Sciences Türkiye, Dr. Suat Seren Chest Disease and Surgery Training and Research Hospital and by the Turkish Ministry of Health, COVID-19 Scientific Research Evaluation Committee (approval date/no: 22.07.2020/49109414-604.02).

The patients who were aged 18 years and over receiving inpatient treatment with the diagnosis of COVID-19 pneumonia between 11.03.2020 and 15.05.2020 were retrospectively included in the study. Patients receiving outpatient treatment were excluded from the study. According to the Guidelines by the Scientific Committee of Ministry, the SARS-CoV-2 real-time reverse-transcriptase-polymerase chain reaction (RT-PCR) test and/or SARS-CoV-2 rapid antibody test was performed for the patients who had a history of contact in the last 14 days and/or symptoms such as cough, fever, and dyspnea (2). RT-PCR test was performed on the date of admission to the hospital from the nasal and pharyngeal area at least once with swab samples. RT-PCR test was repeated for 3 consecutive days for the patients who came back negative. Patients who had positive results were included in the study. Patients given outpatient treatment, patients with negative RT-PCR test and/or SARS-CoV-2 rapid antibody test were excluded from the study.

The patients were grouped according to severity as mild-to-moderate pneumonia, severe pneumonia, and critical disease [i.e. acute respiratory distress syndrome (ARDS), other organ failures, or sepsis]. Mild-to-moderate pneumonia: Respiratory rate <30/minute, SpO_2 >93%, pneumonic infiltration less than 50%. Severe pneumonia: Respiratory rate ⩾30/minute, SpO_2 <90%, patients who had bilateral diffuse pneumonia findings on chest X-ray or tomography. Critical disease: PaO_2/FiO_2<300, SpO_2<90%, hypotension and heart rate >100/minute, acute organ dysfunction development, elevated troponin and arrhythmia, and those with lactate >2 mmol (3).

Patients with COVID-19 pneumonia were divided into 2 groups according to the change in their clinical course during hospital follow-up. Specific criteria were as follows: Progression group: Mild-moderate pneumonia changed to severe pneumonia or critical disease or death; severe pneumonia changed to critical disease or death; critical disease progressed to death. Recovery/stabilization group: Mild-moderate pneumonia, severe pneumonia, and critical disease remained unchanged; mild-moderate pneumonia recovered; severe pneumonia changed to mild-moderate pneumonia; critical-type changed to severe or mild-modreate pneumonia.

In inpatients with COVID-19 pneumonia, the epidemiological and demographic data, contact histories, complaints, habits, comorbidities, initial vital signs, and room air oxygen saturation of the patients were also recorded. Initial hemogram, serum biochemical parameters [i.e. renal and liver functions, lactate dehydrogenase (LDH) and ferritin levels], coagulation profile [i.e. D-dimer, activated partial thromboplastin time, prothrombin time (PT)], myocardial enzymes, C-reactive protein (CRP) values, and treatments

given at the hospitalization were documented from the electronic medical records. Chest radiographs and/or thorax computed tomography (CT) findings were evaluated by a radiologist. The distribution of the lung lesions and the pattern of the lesions were also recorded. The patients were included in one of two groups according to the change in their clinical, laboratory and radiological data during the hospitalization period. The patients who died during the hospitalization period were recorded.

Statistical Analysis

Data were analyzed using the International Business Machines Corporation Statistical Package for the Statistical Package for Social Sciences 22.0 (IBM SPSS Corp.; Armonk, NY, USA) package program. The mean values, standard deviation values, and categorical variables were presented as numbers and percentages. The conformity of continuous variables to the normal distribution was examined by considering graphical research, normality tests, and sampling size. It was found that these variables did not meet the "normal distribution" conditions in all subgroups, and the non-parametric "Mann-Whitney U test" was used for the comparison of the independent groups. The ROC analysis was conducted for the variables that had significant differences and the most appropriate cut-off value was determined according to the Youden index. Dichotomous variables were formed according to these cut-off values. The categorical independent variables are presented in the cross tables as frequencies and percentages, their distributions were compared with the chi-square test, and the univariate odds ratio was calculated. The variables with $p<0.200$ were included in the multivariate logistic regression analysis as independent variables and multivariate odds ratios were calculated with the backward stepwise method (Wald). The margin of error for the first type was determined as α:0.05 and tested as double-tailed in all statistical comparison tests. In the case of $p<0.05$, the difference between the groups was considered statistically significant.

Results

General Characteristics and Clinical Presentations

In this study 233 patients with COVID-19 associated pneumonia included 134 males and 99 females. Median age (minimum-maximum) was 63 (28-91) years in the progression group (n=54) and 52 (20-85) years in the recovery/stabilization group (n=179) (p=0.00). In the progression group, the number of aged ≥65 years, a number of intensive care treatment were significantly higher than the recovery/stabilization group (all p-values <0.05). The progression group had a significantly higher proportion of patients with a history of smoking than the recovery/stabilization group. Frequency of any comorbidity (p=0.007), heart disease, chronic obstructive pulmonary disease (COPD) and malignancy ($p<0.05$) was higher in the progression group than the recovery/stabilization group. Dyspnea and fatigue were more common symptom in the progression group when compared to the recovery/stabilization group (66.7% vs. 32.4%, 57.4% vs. 40.2%, $p<0.05$). In the progression group, the number of patients with body fever >37.5 °C (p=0.042) and blood oxygen saturation ≤93% ($p<0.001$) were higher than the recovery/stabilization group. 57.4% of patients in the progression group (n=31) died (Table 1).

Laboratory Indices and Imaging Characteristics

Laboratory data of patients diagnosed with COVID-19 pneumonia were evaluated at the time of admission. When compared with the recovery/stabilization group, these results showed that leukocyte, neutrophil, neutrophil-lymphocyte ratio (NLR), CRP, LDH, ferritin, D-dimer, PT, international normalized ratio (INR), glucose, creatinine, troponin-T, pro-B-type natriuretic peptide (pro-BNP), and FiO_2 were significantly higher in the progression group than the recovery/stabilization group ($p<0.05$). In addition in the progression group, lymphocyte, monocyte, albumin, and blood oxygen saturation were significantly lower than recovery/stabilization group ($p<0.05$). On the X-ray chest radiography, the bilateral distribution of lesions was significantly more in the progression group than the improvement/stabilization group (84%/63.4%, p=0.012). When the lesion distribution on thorax CT is evaluated; while the lesions mostly showed a central or peripheral distribution in the recovery/stabilization group, the lesions involved all zones (diffuse distribution) in the progression group (p=0.002) (Table 2).

Risk Factors for Disease Progression in Patients with COVID-19 Pneumonia

The risk factors that were found to be significantly associated with the progression of the disease in univariate and multivariate logistic regression analysis are given in Table 3 below.

The Predictors of Progression of COVID-19 Pneumonia Were Determined by ROC Analysis

The sensitivity of NLR was 83.3%, specificity 57.5%, and cut-off >3.545 [area under curve (AUC)=0.752; $p<0.001$], the sensitivity of pro-BNP was 71.8%, specificity 73.8%, and cut-off >332.8 (AUC=0.752; $p<0.001$), the sensitivity of troponin-T was 81.2%, specificity 60.6%, and cut-off >4.58 (AUC=0.730; $p<0.001$) (Figure 1).

Table 1. Demographic data and clinical findings of COVID-19 patients in the progression group and recovery/stabilization group

	Progression group (n=54)	Recovery/stabilization group (n=179)	p
Age (years)	63 (28-91)	52 (20-85)	0.00
Age group ≥65 <65	 19 (35.2) 35 (64.8)	 31 (17.3) 148 (82.7)	0.009
Male gender	37 (68.5%)	97 (54.2%)	0.087
Smoking (pack/year)	33 (5-100)	20 (1-150)	0.019
Smoking status Smoker Ex-smoker Non-smoker	 4 (8) 26 (52) 20 (40)	 34 (19.2) 34 (19.2) 109 (61.6)	0.000
Contact history	9 (16.7)	61 (34.3)	0.021
Inpatient treatment **Inpatient + intensive care treatment** **Intensive care treatment**	21 (38.9) 13 (24.1) 20 (37)	171 (95.5) 6 (3.4) 2 (1.1)	0.000
Any comorbidity	37 (68.5)	83 (46.4)	0.007
Hypertension	18 (33.3)	48 (26.8)	0.448
Diabetes mellitus	11 (20.4)	22 (12.3)	0.180
Cardiac disease	9 (16.7)	12 (6.7)	0.032
COPD	11 (20.4)	10 (5.6)	0.002
Asthma	0 (0)	7 (3.9)	0.358
Malignancy	11 (20.4)	10 (5.6)	0.002
Cough	32 (59.3)	116 (64.8)	0.561
Dyspnea	36 (66.7)	58 (32.4)	0.000
Sputum	9 (16.7)	15 (8.4)	0.133
Headache	6 (11.1)	18 (10.1)	1.00
Weakness	31 (57.4)	72 (40.2)	0.038
Nausea	8 (14.8)	12 (6.7)	0.092
Myalgia	8 (14.8)	41 (22.9)	0.277
Diarrhea	3 (5.6)	12 (6.7)	1.000
Anosmia	1 (1.9)	8 (4.5)	0.689
Heart rate (beats/min)	90 (53-156)	88 (62-140)	0.035
Respiratory rate (breaths/min)	23 (11-36)	18 (10-32)	0.00
Body temperature >37.5 °C	26 (48.1)	57 (31.8)	0.042
Blood oxygen saturation, %	89 (64-99)	95(80-98)	0.00
Blood oxygen saturation ≤93%	39 (72.2)	44 (24.6)	0.00
RT-PCR positivity	51 (94.4)	14 7(82.1)	0.045
Spectrum of disease (severity) Mild-moderate pneumonia Severe pneumonia Critical illness	 35 (64.8) 13 (24.1) 6 (11.1)	 133 (74.3) 28 (15.6) 18 (10.1)	0.326
Time of stay in service (day)	13 (3-27)	7 (3-21)	0.00
Time of stay in the ICU (day)	8 (1-37)	13 (4-30)	0.569
Mortality, n (%)	31 (57.4)	0 (0.00)	0.000

Data are presented as median (interquartile range) or number (%), COPD: Chronic obstructive pulmonary disease, RT-PCR: Reverse transcription-polymerase chain reaction, ICU: Intensive care unit, COVID-19: Coronavirus disease-2019

Table 2. Laboratory findings and imaging characteristics of COVID-19 patients in the progression group and recovery/stabilization group

	Progression group (n=54)	Recovery/stabilization group (n=179)	p
Leukocyte x10³ µL	7850 (13000-31900)	5800 (2600-30900)	0.001
Leukcytosis (>10.000)	17 (31.2)	22 (12.3)	0.002
Neutrophil x10³ µL	6100 (1000-28100)	3800 (1100-30300)	0.00
Lymphocyte x10³ µL	800 (100-3000)	1100 (100-5500)	0.00
Lymphopenia (<800)	23 (42.6)	33 (18.4)	0.001
Monocyte x10³ µL	400 (100-8613)	500 (0-6000)	0.011
NLR	7.42 (1.08-93.67)	3.33 (0.63-75.75)	0.00
Hemoglobin gr/dL	12.9 (8.30-16.7)	13.20 (7.8-17.30)	0.129
Platelet x10³ µL	249500 (65000-649000)	215000 (62000-840000)	0.070
PT (sec)	13.2 (7.48-53.5)	12.5 (10.6-16.9)	0.002
APTT (sec)	26.5 (19.9-95.2)	25.5 (19.8-48.4)	0.342
INR	1.11 (0.89-4.80)	1.04 (0.8-1.44)	0.001
D-dimer, ng/mL	1426 (304-10000)	717 (121-10000)	0.00
D-dimer >1000 ng/mL	35 (71.4)	48 (30.4)	0.00
Albumine, gr/dL	3.19 (1.48-4.10)	3.96 (2.05-5.02)	0.00
Albumine <4 gr/dL	44 (95.7)	74 (52.9)	0.00
Aspartate aminotransferase, U/L	25 (11-97)	23 (10-134)	0.147
Alanine aminotransferase, U/L	21 (4-140)	22 (5-93)	0.764
Total bilurubin, mg/dL	0.41 (0.10-1.47)	0.35 (0.08-2.0)	0.185
Lactate dehydrogenase, U/L	351 (125-969)	228 (97-785)	0.00
Lactate dehydrogenase >243 U/L	32 (71.1)	60 (44.4)	0.003
Glucose mg/dL	125 (57-297)	109 (61-500)	0.008
Glucose ≥120 mg/dL	31 (57.4)	64 (35.8)	0.007
Creatinine, mg/dL	0.96 (0.49-4.10)	0.79 (0.45-2.55)	0.001
C-reactive protein, mg/dL	10.40 (0.07-39)	4.53 (0.08-79.2)	0.00
C-reactive protein >10 mg/dL	29 (53.7)	41 (22.9)	0.00
Ferritin, ng/mL	625 (91-2227)	214 (9-1585)	0.00
Ferritin >500 ng/mL	26 (57.8)	29 (21)	0.00
Troponin-T, ng/L	8.10 (0.0-1664)	3.76 (0.0-105.1)	0.00
Pro-BNP pg/mL	666 (7-36000)	78 (7-8327)	0.00
Blood oxygen saturation, %	89 (64-99)	95 (80-98)	0.00
Blood oxygen saturation ≤93%	39 (72.2)	44 (24.6)	0.00
FiO_2, %	31 (21-200)	21 (21-93)	0.00
pO_2/FiO_2	191 (67-382)	234 (137-331)	0.110
Lesion in X-ray graphic Bilateral Unilateral	 42 (84) 8 (16)	 85 (63.4) 49 (36.6)	0.012
Distribution of lesions on HRCT Central Peripheric Diffuse	 1 (1.9) 18 (34) 34 (64.2)	 8 (4.6) 103 (58.9) 64 (36.6)	0.002

Data are presented as median (interquartile range) or number (%), NLR: Neutrophil-to-lymphocyte ratio, PT: Prothrombin time, aPTT: Activated partial thromboplastin time, INR: International normalized ratio, Pro-BNP: Pro-brain natriuretic peptide, HRCT: High resolution computed tomography, COVID-19: Coronavirus disease-2019

Table 3. Univariate and multivariate analysis results of risk factors for disease progression in COVID-19 patients (n=233)

Univariate analysis				Multivariate analysis		
Variables	**OR**	**95% CI**	**p**	**OR**	**95% CI**	**p**
Age (≥65 years)	2.592	1.314-5.113	0.009			
History of smoking	2.404	1.216-4.56	0.01			
Comorbidity	2.517	1.321-4.798	0.004			
COPD	4.323	1.727-10.843	0.002			
Cardiac disease	2.783	1.104-7.018	0.032			
Cancer	4.323	1.724-10.843	0.002			
Body temperature >37.5 °C	1.987	1.070-3.693	0.042	2.91	1.35-6.30	0.007
Dyspnea	4.172	2.186-7.965	0.00			
Blood oxygen saturation ≤93%	7.977	4.018-15.838	0.00			
Leukcytosis (>10.000)	3.279	1.584-6.785	0.002			
Lymphopenia (<800)	3.283	1.699-6.342	0.001			
NLR >3.55	6.776	3.123-14.703	0.00	4.03	1.71-9.49	0.001
C-reactive protein >10 mg/dL	3.904	2.062-3.793	0.00			
Albumine <4 gr/dL	19.622	4.578-84.101	0.00			
Lactate dehydrogenase >243 U/L	3.077	1.485-6.376	0.003			
Ferritin >500 ng/mL	5.143	2.505-10.561	0.00			
D-dimer >1.000 ng/mL	5.729	2.827-11.612	0.00	3.89	1.75-8.61	0.001
Troponin-T >4.58 ng/L	6.660	2.988-14.849	0.00			
Pro-BNP >333 pg/mL	7.159	2.908-17.627	0.00			

COPD: Chronic obstructive pulmonary disease, NLR: Neutrophil-to-lymphocyte ratio, Pro-BNP: Pro-brain natriuretic peptide, COVID-19: Coronavirus disease-2019

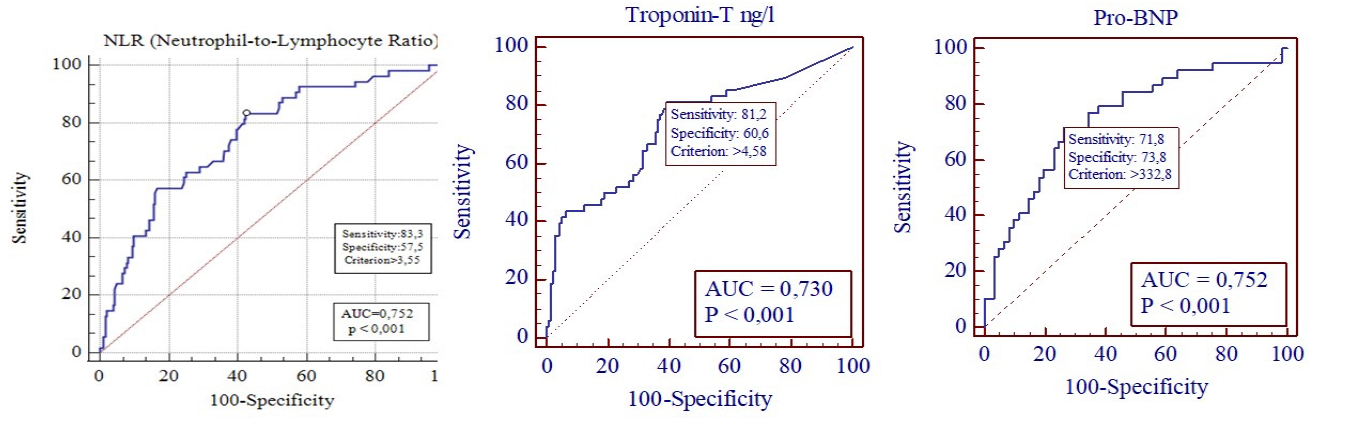

Figure 1. ROC analysis of COVID-19 patients
COVID-19: Coronavirus disease-2019, Pro-BNP: Pro-brain natriuretic peptide, AUC: Area under the curue

Discussion

The clinical course of the disease in COVID-19 infection differs according to age and comorbidities. Severe pneumonia resulting in critical illness and sometimes death may be seen in those with advanced age (>65) and comorbidities. In young people, COVID-19 pneumonia is usually mild to moderate and has been reported to result in recovery (1,4,5). The disease sometimes deteriorates rapidly and can even result in death. Therefore, we planned to investigate the factors predicting disease progression.

In the present study, it was found that the median age, advanced age (≥65 years), smoking and the presence of any comorbidy in the progression group were significantly higher than the recovery/stabilization group (p<0.05). In univariate analysis; it was found that age, smoking, comorbidity, heart disease, COPD, cancer, dyspnea, and fever were risk factors that predicted the disease progression.

In a study in which Lee et al. (6) compared hospitalized mortal and non-mortal advanced-age (≥65 years old) COVID-19 patients, it was reported that male gender, age, and comorbidity were higher in the group that had a mortal group, and advanced age was the most important risk factor for mortality.

Toker et al. (7) retrospectively analyzed 561 COVID-19 patients as intensive care unit (ICU)/non-ICU group and death/survived group. They reported that advanced age, coronary artery disease and malignancy, leukocyte count over ten thousand, lymphopenia, elevation of urea and creatinine, CRP, procalcitonin, LDH, D-dimer and cTnI parameters were significant risk factors for ICU and mortality (7).

Pneumonia and ARDS are the most important and most common COVID-19 complications. During the worsening of the disease, an uncontrolled excessive inflammatory response and subsequent tissue damage are observed. Leukocytes form an important cell group in the systemic inflammatory response in severe disease. Lymphopenia and eosinopenia are also seen (8). The subgroup of leukocytes are used as an index to determine the severity of the immune response. NLR is a biomarker of the systemic inflammatory response (9). Wu et al. (10) found that severe disease was associated with neutrophilia and lymphopenia in COVID-19 patients in the ICU. In another study, neutrophilia and high NLR were found to be correlated with the severity of the disease and poor outcomes (11). In the present study, leukocyte, neutrophil, and NLR were significantly elevated in the progression group when compared to the recovery/stabilization group, and lymphopenia was also significantly more higher. It was determined that laboratory parameters such as leukocytosis, lymphopenia, high NLR, CRP, albumin, LDH, ferritin, D-dimer were the risk factors for disease progression (in univariate analysis). In ROC analysis, the cut-off value of (NLR >3.545) that predicted progression was found in this study. Jimeno et al. (12) found that age, cardiovascular disease, and high CRP and NLR were associated with mortality. The results showed that higher temperature, elevated NLR, and D-dimer were are risk factors for disease progression in multivariate logistic models.

When compared to other viral infections, the risk of venous thromboembolism and pulmonary thromboembolism is higher in severe infections of SARS-CoV-2 (13). Abnormally elevated hypercoagulability markers, increased levels of D-dimer, and thrombocytopenia were also associated with poor prognosis and mortality in COVID-19 (13,14,15). In this study, PT, INR, and D-dimer values were found significantly higher in the progression group, compared to recovery/stabilization group.

In both univariate and multivariate logistic regression analysis, it was determined that elevated D-dimer (>1.000 ng/mL) increased the progression of COVID-19 pneumonia 5.72 and 3.89 times, respectively.

Myocardial damage is associated with the severity and prognosis of the disease in COVID-19 patients (16). In a study, it was conducted on hospitalized patients with COVID-19 diagnosis, troponin-T and NT-pro-BNP levels were found to be significantly higher in patients who died when compared to survivors (17). Selçuk et al. (18) retrospectively analyzed 137 hospitalized patients diagnosed with COVID-19 without heart failure in two groups; mortal and surviving. In multivarite analysis, age, NT-pro BNP, troponin-I, leukocyte, and creatinine levels were associated with in-hospital mortality.

In the ROC analysis, they found the value of NT-pro-BNP predicted in-hospital mortality as 260 ng/L reflecting a sensitivity of 82%, a specificity of 93% (AUC: 0.86; 95% confidence interval: 0.76-0.97) (18).

In the present study, when compared to recovery/stabilization group, it was found that the cardiovascular disease, troponin-T, and pro-BNP levels was significantly higher in patients who progressed and had a mortal. It was determined that troponin-T, pro-BNP and NLR are independently associated with progression in ROC analysis, and that these biomarkers can be used as prognostic factors.

Study Limitations

The study had a narrow design. Larger multicenter studies are needed in this respect. The study was conducted on the data that were obtained in the period when there was no COVID-19 vaccine anywhere in the world. For this reason, no comment could be made on the positive effects of vaccination on the course of the disease and mortality.

Conclusion

Identifying potential risk factors that predict the course of the disease has great importance in reducing morbidity and mortality. We believe that identifying the risk factors that predict the progression of the disease will make a significant contribution to patient-based follow-up and treatment decisions, and, to reducing morbidity and mortality.

Acknowledgments

We would like to thank the COVID study group of University of Health Sciences Türkiye, Dr. Suat Seren Chest Disease and Surgery Training and Research Hospital, Aydan Mertoglu, Bilge Salik, Can Bicmen, Celalettin Yilmaz, Dursun Tatar, Emel Cireli, Fevziye Tuksavul, Gulsum Ari, Gunes Senol, Hulya Sahin, Mehmet Ufuk Yilmaz, Mine Gayaf, Nimet Aksel, Nisel Yılmaz, Ozgur Batum, Ozgur Uslu, Ozlem Ediboglu, Ozlem Yalniz, Pinar Cimen, Sami Cenk Kirakli, Sami Deniz, Semra Bilaceroglu, Serir Ozkan, Serpil Tekgul, Yelda Varol, Zuhre Sarp Taymaz, Ali Kadri Cırak, Berna Komurcuoglu, Berrin Akkol, Burcin Hakoglu, Günseli Balcı, Sena Ataman, Sinem Ermin.

Ethics

Ethics Committee Approval: All procedures performed in studies involving human participants were in accordance with the national ethical standards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics committee of University of Health Sciences Türkiye, Dr. Suat Seren Chest Disease and Surgery Training and Research Hospital, and by the Turkish Ministry of Health, COVID-19 Scientific Research Evaluation Committee (approval date/no: 22.07.2020/49109414-604.02).

Informed Consent: The study was designed retrospectively.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.D.Ü., G.P., D.S.U., Concept: F.D.Ü., G.K., A.A., Design: F.D.Ü., G.P., A.A., E.Y., F.G., Data Collection or Processing: F.D.Ü, G.K., G.P., D.S.U., A.A., E.Y., F.G., Analysis or Interpretation: F.D.Ü., G.K., G.P., Literature Search: F.D.Ü., G.K., D.S.U., E.Y., F.G., Writing: F.D.Ü., G.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases From the Chinese Center for Disease Control and Prevention. JAMA. 2020;323:1239-1242. [Crossref]
2. Ministry of Health.COVID-19 Yeni Koronavirüs Hastalığı. https://covid19bilgi.saglik.gov.tr/ Accessed April 17, 2020. [Crossref]
3. Onder G, Rezza G, Brusaferro S. Case-fatality rate and characteristics of patients dying in relation to COVID-19 in Italy. JAMA. 2020;323:1775-1776. [Crossref]
4. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020;382:1708-1720. [Crossref]
5. Xu XW, Wu XX, Jiang XG, Xu KJ, Ying LJ, Ma CL, et al. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. BMJ. 2020;368:m606. [Crossref]
6. Lee JY, Kim HA, Huh K, Hyun M, Rhee JY, Jang S, et al. Risk Factors for Mortality and Respiratory Support in Elderly Patients Hospitalized with COVID-19 in Korea. J Korean Med Sci. 2020;35:e223. [Crossref]
7. Toker AK, Kılıç AU, Eren E, Dursun ZB, Toker İ, Saatçi E, et al. Confronting a Pandemic in Early Stages: A Retrospective Analysis From a Pandemic Hospital. Hamidiye Med J. 2020;1:106-118. [Crossref]
8. Anka AU, Tahir MI, Abubakar SD, Alsabbagh M, Zian Z, Hamedifar H, et al. Coronavirus disease 2019 (COVID-19): An overview of the immunopathology, serological diagnosis and management. Scand J Immunol. 2021;93:e12998. [Crossref]
9. Zahorec R. Ratio of neutrophil to lymphocyte counts--rapid and simple parameter of systemic inflammation and stress in critically ill. Bratisl Lek Listy. 2001;102:5-14. [Crossref]
10. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020;579:265-269. [Crossref]
11. Cao X. COVID-19: immunopathology and its implications for therapy. Nat Rev Immunol. 2020;20:269-270. [Crossref]
12. Jimeno S, Ventura PS, Castellano JM, García-Adasme SI, Miranda M, Touza P, et al. Prognostic implications of neutrophil-lymphocyte ratio in COVID-19. Eur J Clin Invest. 2021;51:e13404. [Crossref]
13. Tang N, Bai H, Chen X, J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. J Thromb Haemost. 2020;18:1094-1099. [Crossref]
14. Thachil J, Tang N, Gando S, Falanga A, Cattaneo M, Levi M, et al. ISTH interim guidance on recognition and management of coagulopathy in COVID-19. J Thromb Haemost. 2020;18:1023-1026. [Crossref]
15. Xiang-Hua Y, Le-Min W, Ai-Bin L, Zhu G, Riquan L, Xu-You Z, et al. Severe acute respiratory syndrome and venous thromboembolism in multiple organs. Am J Respir Crit Care Med. 2010;182:436-437.
16. Tajbakhsh A, SM, H, Akbari A, Inabadi M, Savardashtak A, et al. COVID-19 and cardiac injury: clinical manifestations, biomarkers, mechanisms, diagnosis, treatment, and follow up. Expert Rev Anti Infect Ther. 2021;19:345-357. [Crossref]
17. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, et al. Cardiovascular Implications of Fatal Outcomes of Patients With Coronavirus Disease 2019 (COVID-19). JAMA Cardiol. 2020;5:811-818. [Crossref]
18. Selçuk M, Keskin M, Çınar T, Günay N, Doğan S, Çiçek V, et al. Prognostic significance of N-Terminal Pro-BNP in patients with COVID-19 pneumonia without previous history of heart failure. J Cardiovasc Thorac Res. 2021;13:141-145. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2022.92063

Hamidiye Med J 2022;3(2):132-139

The Role of Inflammatory Parameters in the Prognosis of Patients with COVID-19

COVID-19 Hastalarında Enflamatuvar Parametrelerin Prognostik Rolü

Kadir Canoğlu, Tayfun Çalışkan, Ecem Sinmez, Ömer Ayten

University of Health Sciences Türkiye Hamidiye Faculty of Medicine; İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital, Clinic of Pulmonology, İstanbul, Türkiye

ABSTRACT

Background: The prognostic significance of inflammatory parameters in patients with Coronavirus disease-2019 (COVID-19) has been investigated.

Materials and Methods: Neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio, C-reactive protein (CRP) albumin ratio (CAR), systemic inflammation index (SII), CRP-lymphocyte ratio (CRP/L), neutrophil to lymphocyte, platelet ratio (NLPR), red cell volume distribution width index (RDW-SD), and procalcitonin were evaluated in relation to admission to intensive care unit (ICU) and mortality in 419 patients with moderate-to-severe COVID-19.

Results: NLR, CAR, CRP/L, NLPR, RDW-SD and procalcitonin levels were higher both in those who needed ICU compared to those who did not (p=0.001, 0.005, 0.002, 0.001, 0.001 and 0.001; respectively), and in those who died compared to the survival group (p=0.001, 0.024, 0.009, 0.001, 0.001 and 0.001; respectively). SII was higher only in those who needed ICU (p=0.001). NLR (0.610, p=0.002), CAR (0.602, p=0.005), SII (0.573, p=0.043), CRP/L (0.593, p=0.010), and NLPR (0.618, p=0.001) were statistically significant for admission to ICU; and NLR (0.637, p=0.006), CAR (0.613, p=0.024), CRP/L (0.605, p=0.035) and NLPR (0.660, p=0.001) were statistically significant for mortality in the evaluation of area under curve in ROC analysis. RDW-SD was an independent risk factor for both ICU admission [odds ratio (OR): 1.194, p=0.024] and mortality (OR: 1.263, p=0.002), and procalcitonin was an independent risk factor for ICU admission (OR: 1.492, p=0.034) in multivariate analysis.

Conclusion: NLR, CAR, CRP/L, NLPR, RDW-SD and procalcitonin were determined as prognostic parameters in terms of both the need for ICU and mortality in patients with COVID-19. SII was a prognostic parameter only for the need for ICU.

Keywords: COVID-19, inflammatory index, intensive care unit, lymphocytes, mortality

ÖZ

Amaç: Koronavirüs hastalığı-2019 (COVID-19) hastalarında enflamatuvar parametrelerin prognostik önemi araştırılmıştır.

Gereç ve Yöntemler: Dört yüz on dokuz orta-ağır COVID-19 hastasında, nötrofil lenfosit oranı (NLR), platelet lenfosit oranı, C-reaktif protein (CRP) albümin oranı (CAR), sistemik enflamasyon indeks (SII), CRP lenfosit oranı (CRP/L), nötrofil-lenfosit, platelet oranı (NLPR), kırmızı hücre volüm dağılım genişliği (RDW-SD) ve prokalsitoninin yoğun bakım ünitesine (YBÜ) giriş ve mortalite ile ilişkisi değerlendirilmiştir.

Bulgular: NLR, CAR, CRP/L, NLPR, RDW-SD, prokalsitonin hem YBÜ'ye girenlerde girmeyenlere göre artış (sırasıyla p=0,001, 0,005, 0,002, 0,001, 0,001 ve 0,001), hem de ölenlerde survival grubuna göre artış (sırasıyla p=0,001, 0,024, 0,009, 0,001, 0,001 ve 0,001) saptandı. SII sadece yoğun bakıma girenlerde artış saptandı (p=0,001). ROC analizinde eğri altındaki alan değerlendirmesinde, YBÜ'ye giriş için NLR (0,610, p=0,002), CAR (0,602, p=0,005), SII (0,573, p=0,043), CRP/L (0,593, p=0,010), NLPR (0,618, p=0,001); mortalite için NLR (0.637, p=0,006), CAR (0,613, p=0,024), CRP/L (0,605, p=0,035), NLPR (0,660, p=0,001) saptandı. Multivaryant analizde, RDW-SD hem YBÜ girişi [olasılık oranı (OO): 1,194, p=0,024] hem de mortalite için (OO: 1,263, p=0,002), prokalsitonin de YBÜ'ye giriş için (OO: 1,492, p=0,034) bağımsız risk faktörü olarak saptanmıştır.

Sonuç: NLR, CAR, CRP/L, NLPR, RDW-SD ve prokalsitonin COVID-19 hastalarında hem YBÜ hem de mortalite için prognostik önemli olabilecek parametreler olarak değerlendirilmiştir. SII ise sadece YBÜ için prognostik önemi olabileceği saptanmıştır.

Anahtar Kelimeler: COVID-19, enflamatuvar indeks, yoğun bakım, lenfosit, mortalite

Address for Correspondence: Kadir Canoğlu, University of Health Sciences Türkiye Hamidiye Faculty of Medicine; İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital, Clinic of Pulmonology, İstanbul, Türkiye

Phone: +90 216 542 20 20 E-mail: kadircano@gmail.com **ORCID ID:** orcid.org/0000-0003-1579-3392

Received: 25.04.2022 **Accepted:** 14.06.2022

©Copyright 2022 by University of Health Sciences Türkiye Hamidiye Faculty of Medicine. / Hamidiye Medical Journal published by Galenos Yayınevi.

Introduction

The Coronavirus disease-2019 (COVID-19) epidemic has affected the whole world, causing approximately 300 million confirmed cases and 5.5 million deaths from December 2019 to January 2022 (1). Its clinical spectrum ranges from asymptomatic and mild to critical illness including acute respiratory distress syndrome (ARDS), multi-organ failure and death (2). The severe clinical condition elicited by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) is due to ARDS, macrophage activation, cytokine storm, endothelial dysfunction and coagulopathy caused by immune system dysfunction and hyperinflammation (3).

Whole blood parameters, white blood cell and sub-parameters including neutrophils, lymphocytes, monocytes, eosinophils and basophils are inexpensive and easily accessible biomarkers of systemic inflammation. Neutrophils usually increase and lymphocytes decrease in the progression of inflammatory diseases (4). Platelets play a key role in hemostasis. It also plays a role in host defense in infection and is an effector and modulator of immune cells (5). While whole blood parameters and other inflammation parameters have roles alone in demonstrating systemic inflammation, parameters resulting from their ratio to each other can also be used.

Parameters such as neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), systemic inflammation index (SII), monocyte to lymphocyte ratio, derived NLR (dNLR), mean platelet volume to platelet ratio (MPR), lymphocyte to monocyte ratio (LMR), C-reactive protein (CRP) albumin ratio (CAR), CRP lymphocyte ratio (CRP/L), neutrophil to lymphocyte, platelet ratio (NLPR), systemic inflammation response index (SIRI) and aggregate index of systemic inflammation (AISI) have been studied in many inflammatory diseases and cancers and also the role of them in the diagnosis, prognosis and follow-up of patients with COVID-19 has also been studied (6,7,8,9,10).

The aim of this study was to investigate the role of different inflammatory parameters (including NLR, PLR, CAR, SII, NLPR, CRP/L, procalcitonin) in predicting intensive care unit (ICU) admission and mortality in hospitalized patients with moderate and severe COVID-19.

Material and Methods

Hospitalized patients with moderate-to-severe COVID-19 pneumonia were included in this retrospective, single-center study. 419 patients, over 18 years old, who were hospitalized in University of Health Sciences Türkiye, İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital, İstanbul, Türkiye between March 15, 2020 and September 30, 2020 were included in the study. Informed consent was waived because of the retrospective nature of the study. This study was approved by the Ethics Committee of the Ümraniye Training and Research Hospital (date: November 19, 2020, no: 346). Patients with malignancy, immunosuppression, mild COVID-19 disease [patients with real-time reverse transcriptase polymerase chain reaction (RT-PCR) positive but no lung involvement], those younger than 18 years of age, and outpatient COVID-19 patients were not included in this study. Patients who needed ICU less than 5 days after hospitalization were excluded from the study. SARS-CoV-2 was founded by RT-PCR using a COVID-19 Nucleic Acid Detection Kit according to the manufacturer's recommendation (Bioeksen Ltd., Türkiye). Lung involvement was detected by chest X-ray and, if necessary, by thorax computed tomography.

Moderate and severe disease definitions were determined according to the World Health Organization (WHO) weight classification and the COVID-19 guidelines of the Turkish Ministry of Health (11,12). Severe COVID-19 was defined in the presence of oxygen saturation (SpO_2) <90% in room air, bilateral diffuse lung involvement, tachypnea >30/min, no need for invasive mechanical ventilation, and not presenting with ARDS, sepsis, or septic shock. Moderate COVID-19 was determined as mild lung involvement and absence of severe COVID-19 findings. Three hundred nine (73.7%) of the patients included in this study were moderate cases and 110 (26.3%) were severe cases. All patients were treated with favipiravir in accordance with the Turkish national COVID-19 guidelines. Oxygen support was given to patients with SpO_2 <90% in room air. Antibiotic treatment was given to patients with suspected or proven secondary bacterial infection. Steroid therapy was given to patients with worsening hypoxemia and increased lung involvement, and patients who developed macrophage activation syndrome were treated with anti-IL6 (tocilizumab) therapy according to the WHO and Türkiye national COVID-19 guidelines (11,12). Patients with septic shock, hypotension persisting despite fluid support and requiring vasopressor therapy, ARDS, and the need for non-invasive and invasive mechanical ventilation were admitted to the ICU.

Patients were classified as non-survivor and survivor; and needing ICU and not needing ICU. Patients' age, gender, hematocrit (HCT), white cell cells (WBC), mean corpuscular volume (MCV), red cell volume distribution width index (RDW-SD), platelet, neutrophil, lymphocyte, CRP, procalcitonin, D-dimer, albumin, and ferritin were compared between these groups. Different inflammatory parameters calculated from the parameters measured in whole blood were also compared between the groups. The calculation of these inflammatory parameters is as follows;

NLR (neutrophil/lymphocyte), PLR (platelet/lymphocyte), CAR (CRP/albumin), SII [(neutrophilxplatelet)/lymphocyte], CRP/L (CRP/lymphocyte), NLPR (neutrophil/[lymphocytex platelet]).

Statistical Analysis

Descriptive analyzes (frequency distributions, percentage, mean, median and interquartile range) were used as statistical methods in the analysis of the data in the study. Normality of the data was ensured by Kolmogorov-Smirnov test. The t-test was used for those with normal distribution, and the Mann-Whitney U test was used for continuous variables. Logistic regression analysis was used to calculate influencing factors, ROC curve analysis was used for cut-off point, and sensitivity analysis was used for sensitivity. The results were evaluated at the 95% confidence interval (CI), at the $p<0.05$ significance level. In the analysis of the data, PSPP (PSPP is free software; you can redistribute it and/or modify it under the terms of the GNU General Public License as published by the Free Software Foundation; either version 3 of the License, or (at your option any later version) and Microsoft Excel computer programs were used.

Results

The study included 419 hospitalized patients with moderate and severe COVID-19. One hundred sixty-six (39.62%) of the patients were female and 253 (60.38%) were male. The median age of the patients was calculated as 59 years (range; 19-98). Eighty-seven (20.76%) patients needed ICU and 41 (9.80%) patients died. The patients were compared by dividing them into groups as those who needed ICU and those who did not, and those who died and survived. The parameters and factors affecting mortality and ICU admission were tried to be determined.

Comparison of Patient Groups with and without ICU Admission

There was a statistically significant difference between the ages of patients with ICU admission and no ICU admission [66 (37-98) vs. 57 (19-96) years; respectively, p=0.001]. Fifty-four (62.07%) of the 87 patients admitted to the ICU were male and 33 (37.93%) were female. There was no statistical difference between the groups in terms of gender (p=0.72). Similarly, there was no statistically significant difference between the two groups for WBC, Hb, HCT, platelet and D-dimer levels (p=0.081, 0.118, 0.055, 0.156 and 0.120, respectively). MCV was higher in the ICU admission group than in the no ICU admission group [86.4 (63.6-96.4) fL vs. 85.65 (8.4-102.9) fL; respectively, p=0.019]. RDW-SD was significantly higher in the ICU admission group [13.5 (11.8-25.3)] than in the no ICU admission group [13.1 (11.4-36.1)] (p=0.001). The number of neutrophils was higher in the ICU admission group than in the no ICU admission group [5.2 (1.94-41.18) x 10^3/μL vs. 4.42 (1.48-18.35) x 10^3/μL; respectively, p=0.008]. The lymphocyte count was significantly lower in the ICU admission group compared to the no ICU admission group [1.09 (0.23-3.1) x 10^3/μL vs. 1.14 [0.36-229] x 10^3/μL; respectively, p=0.031). Albumin was statistically lower in the ICU admission group than in the no ICU admission group [32 (22-44) g/L vs 35 (21-47) g/L; respectively, p=0.001]. CRP was 83.8 (6.3-311.9) mg/L in the ICU admission group and 62.1 (2-270.4) mg/L in the no ICU admission group, and the difference between the groups was statistically significant (p=0.004). Similarly, ferritin was significantly higher in the ICU admission group compared to the no ICU admission group [671.72 (41.94-3152.33) ng/mL vs. 388.21 (5.39-3406.07) ng/mL; respectively, p=0.001]. Procalcitonin was 0.18 (0.01-9.42) ng/mL in the ICU admission group and 0.05 (0-9.41) ng/mL in the no ICU admission group, the difference was statistically significant (p=0.001) (Table 1).

NLR was significantly higher in the ICU admission group compared to the no ICU admission group [4.63 (1.31-31.87) vs. 3.66 (0.02-21.8); respectively, p=0.001] in the evaluation of inflammation parameters. CAR [2.49 (0.18-14.18) vs. 1.88 (0.05-8.18); respectively, p=0.005], SII [860.57 (193.17-5931.03) vs. 702.35 (6.29-7280); respectively, p=0.020], CRP/ L [81.93 (7.29-1300.87) vs. 51.68 (0.32-521.91); respectively, p=0.002] and NLPR [0.03 (0.01-0.23) vs. 0.02 (0-0.44); respectively, p=0.001], were significantly higher in ICU admission group than the no ICU admission group. There was no statistically significant difference between the two groups in terms of PLR (p=0.313) (Table 1).

ROC curve analysis was performed to evaluate the prediction of ICU admission in patients with COVID-19. Sensitivity, specificity and area under the ROC curve (AUC) for NLR were 61%, 51% and 0.610, respectively at a cut-off (4.5); and sensitivity, specificity and AUC for CAR were 61%, 49% and 0.602, respectively at a cut-off (2.5). Similarly, sensitivity, specificity and AUC for SII were 56%, 53% and 0.573, respectively at a cut-off (800); and 55%, 56% and 0.593 for CRP/L, respectively at a cut-off (60); and 64%, 52% and 0.618 for NLPR, respectively at a cut-off (0.026) (Table 2 and Figure 1A). In Univariate regression analysis, there was statistically significant relationship between, RDW-SD (OR: 1.242, p=0.008), procalcitonin (OR: 1.579, p=0.011), NLR (OR: 1.104, p=0.000), CAR (OR: 1.202, p=0.001), SII (OR: 1.000, p=0.011), CRP/L (OR: 1.004, p=0.002), NLPR (OR: 2.393, p=0.000) and the need for admission to ICU but not for PLR (OR: 1.001, p=0.165). RDW-SD (OR: 1.194, p=0.024) and procalcitonin (OR: 1.492, p=0.034) were defined as

Table 1. Characteristics of patients with COVID-19 by ICU admission status

	ICU admission (n=87)	No ICU admission (n=332)	p
Age	66 (37-98)	57 (19-96)	**0.001**
Gender Male Female	 54 (62.07%) 33 (37.93%)	 199 (59.94%) 133 (40.06%)	 0.72
WBC (10^3/μL)	6.83 (3.32-45.6)	6.15 (2.26-20.22)	0.081
Hb (g/dL)	13.2 (9.1-15.9)	13.5 (9.5-43.7)	0.118
HCT (%)	38.9 (21.1-50.3)	40.15 (15.9-51.9)	0.055
MCV (fL)	86.4 (63.6-96.4)	85.65 (8.4-102.9)	**0.019**
RDW-SD	13.5 (11.8-25.3)	13.1 (11.4-36.1)	**0.001**
Platelets (10^3/μL)	176 (38-463)	195 (12.7-633)	0.156
Neutrophil (10^3/μL)	5.2 (1.94-41.18)	4.42 (1.48-18.35)	**0.008**
Lymphocyte (10^3/μL)	1.09 (0.23-3.1)	1.14 (0.36-229)	**0.031**
CRP (mg/L)	83.8 (6.3-311.9)	62.1 (2-270.4)	**0.004**
D-dimer (μg/mL)	0.51 (0.01-8.66)	0.4 (0.01-702)	0.120
Albumin (g/L)	32 (22-44)	35 (21-47)	**0.001**
Ferritin (ng/mL)	671.72 (41.94-3152.33)	388.21 (5.39-3406.07)	**0.001**
Procalcitonin (ng/mL)	0.18 (0.01-9.42)	0.05 (0-9.41)	**0.001**
NLR	4.63 (1.31-31.87)	3.66 (0.02-21.8)	**0.001**
PLR	166.21 (43.68-629.17)	165.6 (1.15-794.03)	0.313
CAR	2.49 (0.18-14.18)	1.88 (0.05-8.18)	**0.005**
SII	860.57 (193.17-5931.03)	702.35 (6.29-7280)	**0.020**
CRP/L	81.93 (7.29-1300.87)	51.68 (0.32-521.91)	**0.002**
NLPR	0.03 (0.01-0.23)	0.02 (0-0.44)	**0.001**

Data are median (IQR); n (%). WBC: White blood cell, Hb: Hemoglobin concentration, HCT: Hematocrit value, MCV: Mean corpuscular volume, RDW-SD: Red cell volume distribution width index, CRP: C-reactive protein, NLR: Neutrophil to lymphocyte ratio, PLR: Platelet to lymphocyte ratio, CAR: C-reactive protein to albumin ratio, SII: Systemic inflammation index, CRP/L: C-reactive protein to lymphocyte ratio, NLPR: Neutrophil to lymphocyte x platelet ratio, COVID-19: Coronavirus disease-2019, ICU: Intensive care unit

independent risk factors in predicting admission to ICU in multivariate analysis (Table 3).

Comparison of Non-survivor and Survivor Patient Groups

There was a statistically significant difference between the ages of the patients in the non-survivor and survivor groups [72 (55-98) vs. 57.5 (19-96) years; respectively, p=0.001]. Twenty-nine (70.73%) of the 41 patients who died were male and 12 (29.27%) were female, and there was no statistical difference between the groups in terms of gender (p=0.154). Similarly, there was no significant difference between the two groups for WBC, Hb, HCT and D-dimer levels (p=0.110, 0.113, 0.087 and 0.077, respectively). MCV was significantly higher in the non-survivor group than in the survivor group [87 (63.6-96.4) fL vs. 85.7 (8.4-102.9) fL; respectively, p=0.030]. RDW-SD was significantly higher in the non-survivor group [14 (11.8-25.3)] compared to the survivor group [13.1 (11.4-36.1) (p=0.001)]. Platelet count [167 (38-411) x 10^3/μL vs. 193.5 (12.7-633) x 10^3/μL; respectively, p=0.039], neutrophil count [5.38 (2.24-41.18) x 10^3/μL vs. 4.53 (1.48-18.35) x 10^3/μL; respectively, p=0.025], CRP [96.8 (6.3-299.2) mg/L vs. 64.8 (2-311.9) mg/L; respectively, p=0.016], ferritin [716.32 (86.46-3064.63) ng/mL vs. 390.56 (5.39-3406.07) ng/mL; respectively, p=0.008] and procalcitonin [0.25 (0.01-7.12) ng/mL vs. 0.06 (0-9.42) ng/mL; respectively, p=0.001] were significantly higher in the non-survivor group than in the survivor group. Lymphocyte count [0.96 (0.23-3.1) x 10^3/μL vs. 1.15 (0.36-229) x 10^3/μL; respectively, p=0.019] and albumin [31 (23-43) g/L vs. 35 (21-47) g/L; respectively, p=0.008] were significantly lower in the non-survivor group compared to the survivor group (Table 4).

NLR was significantly higher in the non-survivor group compared to the survivor group [6.32 (1.72-31.87) vs. 3.76 (0.02-21.8); respectively, p=0.001] in the evaluation of inflammation parameters. Similarly, CAR [2.48 (0.18-10.98) vs. 1.94 (0.05-14.18); respectively, p=0.024], CRP/L [86.58 (7.29-1300.87) vs. 55.35 (0.32-521.91); respectively,

p=0.009] and NLPR [0.03 (0.01-0.23) vs. 0.02 (0-0.44); respectively, p=0.001] were significantly higher in the non-survivor group compared to the survivor group. There was no significant difference between the two groups in terms of PLR and SII (p=0.501, 0.064; respectively) (Table 4).

ROC curve analysis was performed to evaluate predicting mortality in patients with COVID-19. Sensitivity, specificity and AUC for NLR were 60%, 59% and 0.637, respectively at a cut-off (4.5); and 59%, 49% and 0.613 for CAR, respectively at a cut-off (2.5). The sensitivity, specificity and AUC for CRP/L were 54%, 63% and 0.605, respectively at a cut-off (60); and 63%, 61% and 0.660 for NLPR, respectively at a cut-off (0.026) (Table 2 and Figure 1B).

Table 2. ROC curve analysis for the ICU admission and mortality of different inflammation parameters in patients with COVID-19

ICU admission							
	AUC	**Cut-off**	**Sensitivity**	**Specificity**	**p**	**95% CI lower**	**95% CI upper**
NLR	0.610	4.5	61%	51%	**0.002**	0.542	0.677
PLR	0.530	-	-	-	0.398	0.459	0.602
CAR	0.602	2.5	61%	49%	**0.005**	0.535	0.669
SII	0.573	800	56%	53%	**0.043**	0.502	0.643
CRP/L	0.593	60	55%	56%	**0.010**	0.527	0.659
NLPR	0.618	0.026	64%	52%	**0.001**	0.554	0.682
Mortality							
	AUC	**Cut-off**	**Sensitivity**	**Specificity**	**p**	**95% CI lower**	**95% CI upper**
NLR	0.637	4.5	60%	59%	**0.006**	0.537	0.736
PLR	0.521	-	-	-	0.671	0.416	0.627
CAR	0.613	2.5	59%	49%	**0.024**	0.518	0.708
SII	0.575	-	-	-	0.136	0.472	0.677
CRP/L	0.605	60	54%	63%	**0.035**	0.514	0.697
NLPR	0.660	0.026	63%	61%	**0.001**	0.569	0.752

CI: Confidence interval, NLR: Neutrophil to lymphocyte ratio, PLR: Platelet to lymphocyte ratio, CAR: C-reactive protein to albumin ratio, SII: Systemic inflammation index, CRP/L: C-reactive protein to lymphocyte ratio, NLPR: Neutrophil to lymphocyte x platelet ratio, COVID-19: Coronavirus disease-2019, ICU: Intensive care unit, AUC: Area under curve

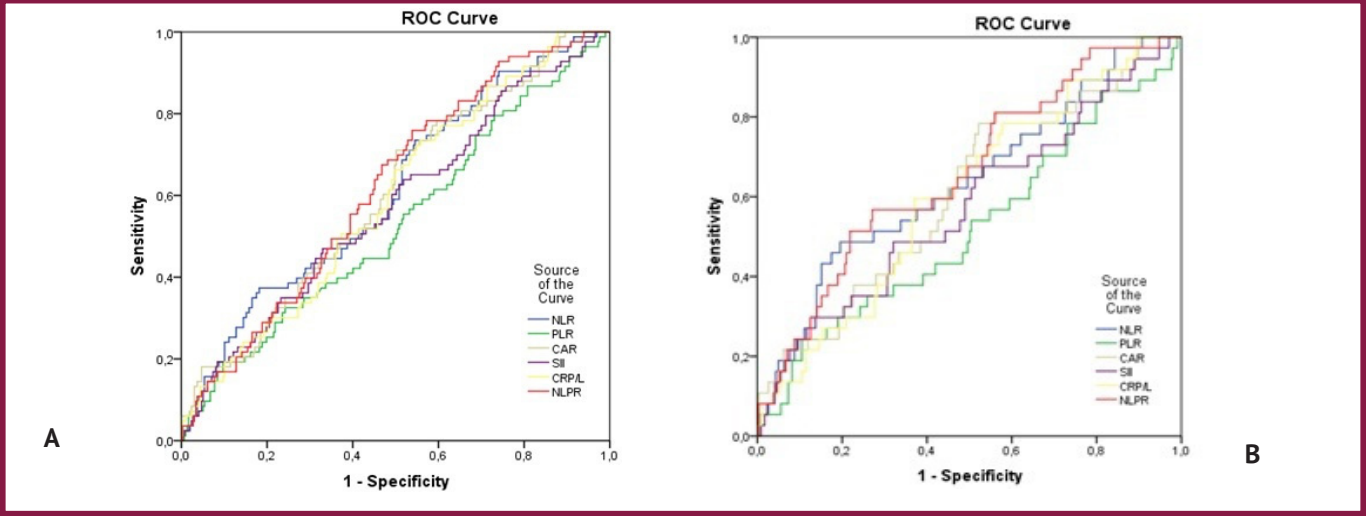

Figure 1. The ROC curve results for the (A) ICU admission and (B) mortality of NLR, PLR, CAR, SII, CRP/L and NLPR

NLR: Neutrophil to lymphocyte ratio, PLR: Platelet to lymphocyte ratio, CAR: C-reactive protein to albumin ratio; SII: Systemic inflammation index, CRP/L: C-reactive protein to lymphocyte ratio, NLPR: Neutrophil to lymphocyte, platelet ratio

Table 3. Univariate and multivariate logistic regression with ICU admission and mortality in patients with COVID-19

	Univariate logistic regression							
	ICU admission				Mortality			
	OR	95% CI lower	95% CI upper	p	OR	95% CI lower	95% CI upper	p
RDW-SD	1.242	1.059	1.456	**0.008**	1.310	1.093	1.570	**0.003**
Procalcitonin (ng/mL)	1.579	1.113	2.241	**0.011**	1.400	1.088	1.803	**0.009**
NLR	1.104	1.047	1.165	**0.000**	1.128	1.060	1.200	**0.000**
PLR	1.001	0.999	1.004	0.165	1.002	0.999	1.004	0.239
CAR	1.202	1.076	1.343	**0.001**	1.221	1.063	1.403	**0.005**
SII	1.000	1.000	1.000	**0.011**	1.000	1.000	1.001	**0.017**
CRP/L	1.004	1.001	1.006	**0.002**	1.004	1.001	1.007	**0.007**
NLPR	2.393	1.858	3.083	**0.000**	1.128	1.060	1.200	**0.000**
	Multivariate logistic regression							
	ICU admission				Mortality			
	OR	95% CI lower	95% CI upper	p	OR	95% CI lower	95% CI upper	p
RDW-SD	1.194	1.024	1.392	**0.024**	1.263	1.089	1.464	**0.002**
Procalcitonin (ng/mL)	1.492	1.031	2.160	**0.034**	-	-	-	-

ICU: Intensive care unit, OR: Odds ratio, CI: Confidence interval, RDW-SD: Red cell volume distribution width index, NLR: Neutrophil to lymphocyte ratio, PLR: Platelet to lymphocyte ratio, CAR: C-reactive protein to albumin ratio, SII: Systemic inflammation index, CRP/L: C-reactive protein to lymphocyte ratio; NLPR: Neutrophil to lymphocyte x platelet ratio, COVID-19: Coronavirus disease-2019

Table 4. Characteristics of patients with COVID-19 by survival status

	Non-survivor (n=41)	Survivor (n=378)	p
Age	72 (55-98)	57.5 (19-96)	**0.001**
Gender Male Female	 29 (70.73%) 12 (29.27%)	 224 (59.26%) 154 (40.74%)	 0.154
WBC (10^3/µL)	7.03 (3.68-45.67)	6.22 (2.26-20.22)	0.110
Hb (g/dL)	13 (9.1-15.7)	13.5 (9.5-43.7)	0.113
HCT (%)	38.7 (28.1-50.3)	40 (15.9-51.9)	0.087
MCV (fL)	87 (63.6-96.4)	85.7 (8.4-102.9)	**0.030**
RDW-SD (%)	14 (11.8-25.3)	13.1 (11.4-36.1)	**0.001**
Platelets (10^3/µL)	167 (38-411)	193.5 (12.7-633)	**0.039**
Neutrophil (10^3/µL)	5.38 (2.24-41.18)	4.53 (1.48-18.35)	**0.025**
Lymphocyte (10^3/µL)	0.96 (0.23-3.1)	1.15 (0.36-229)	**0.019**
CRP (mg/L)	96.8 (6.3-299.2)	64.8 (2-311.9)	**0.016**
D-dimer (µg/mL)	0.52 (0.01-8.66)	0.41 (0.01-702)	0.077
Albumin (g/L)	31 (23-43)	35 (21-47)	**0.008**
Ferritin (ng/mL)	716.32 (86.46-3064.63)	390.56 (5.39-3406.07)	**0.008**
Procalcitonin (ng/mL)	0.25 (0.01-7.12)	0.06 (0-9.42)	**0.001**
NLR	6.32 (1.72-31.87)	3.76 (0.02-21.8)	**0.001**
PLR	166.21 (43.68-629.17)	165.6 (1.15-794.03)	0.501
CAR	2.48 (0.18-10.98)	1.94 (0.05-14.18)	**0.024**
SII	1025.45 (193.17-5797.09)	716.03 (6.29-7280)	0.064
CRP/L	86.58 (7.29-1300.87)	55.35 (0.32-521.91)	**0.009**
NLPR	0.03 (0.01-0.23)	0.02 (0-0.44)	**0.001**

Data are median (IQR); n (%). WBC: White blood cell, Hb: Hemoglobin concentration, HCT: Hematocrit value, MCV: Mean corpuscular volume, RDW-SD: Red cell volume distribution width index, CRP: C-reactive protein, NLR: Neutrophil to lymphocyte ratio, PLR: Platelet to lymphocyte ratio, CAR: C-reactive protein to albumin ratio, SII: Systemic inflammation index, CRP/L: C-reactive protein to lymphocyte ratio, NLPR: Neutrophil to lymphocyte x platelet ratio, COVID-19: Coronavirus disease-2019

In univariate regression analysis, there was a statistically significant relationship between, RDW-SD (OR: 1.310, p=0.003), procalcitonin (OR: 1.400, p=0.009), NLR (OR: 1.128, p=0.000), CAR (OR: 1.221, p=0.005), SII (OR: 1.000, p=0.017), CRP/L (OR: 1.004, p=0.007), NLPR (OR: 1.128, p=0.000) and mortality but not for PLR (OR: 1.002, p=0.239). RDW-SD (OR: 1.263, p=0.002) was defined as an independent risk factor in predicting mortality in multivariate analysis (Table 3).

Discussion

COVID-19 that affects the whole world has a clinical spectrum ranging from asymptomatic disease to critical cases. ARDS, severe pneumonia and multiorgan failure which may require admission to the ICU and result in death may be seen in severe cases (13). The incidence of ARDS and severe disease was 14.8% and 18.1%, respectively, and the fatality rate was 4.3% in the meta-analysis by Sun et al. (14). This demonstrates the importance of parameters that can predict poor outcome in patients with COVID-19. It is known that indices calculated by whole blood parameters alone or by their ratios to each other can be used to show systemic inflammation in COVID-19 and many cancers (9). This study was aimed to evaluate the value of inflammation parameters in predicting ICU admission and mortality in patients with COVID-19.

Higher WBC and neutrophil levels and lower lymphocyte and platelet levels were associated with mortality in the meta-analysis of Taylor et al. (15). High CRP levels were associated with the severity of the disease in another meta-analysis by Meng et al. (16). In this study, similar to the literature, high neutrophil and CRP levels, low lymphocyte levels were associated with ICU admission and mortality in patients with COVID-19. On the other hand, platelet was associated only with mortality. In contrast, there was no association between WBC and prognosis.

Many diseases affect the morphology and functions of red blood cells. Viral infections can affect red blood cells, changing their size, firmness and distribution width. This change was also detected in patients with COVID-19 and was associated with the severity of the disease (17). RDW-SD and MCV were associated with mortality, while Hb and HCT were not in the study of Pluta et al. (18) including 70 patients who were admitted to the ICU with COVID-19. Similarly, in this study, Hb and HCT were ineffective for prognosis, while MCV and RDW-SD were associated with both mortality and ICU admission. In addition, RDW-SD was shown to be an independent risk factor for both mortality (OR: 1.194, p=0.024) and admission to ICU (OR: 1.263, p=0.002) in the multivariate analysis.

It was stated that high D-dimer and low albumin levels were associated with mortality in a meta-analysis by Wu et al. (19). Low albumin was associated with both ICU admission and mortality in our study. On the other hand, D-dimer was high in the evaluation of both groups, but there was no statistically significant difference.

In our study, the need for ICU and mortality risk were higher when NLR >4.5, CAR >2.5, CRP/L >60, NLPR >0.026. When SII was >800, there was a statistically significant increase in those admitted to the ICU, but there was no correlation between it and mortality. There was no relationship between both ICU admission and mortality and PLR. In addition, NLR, CAR, SII, CRP/L and NLPR were associated with both ICU admission and mortality in the univariate analysis. ROC analysis was performed for more detailed information, and AUC was 0.610, 0.602, 0.573, 0.593 and 0.618 at admission to ICU for NLR, CAR, SII, CRP/L and NLPR, respectively. The AUC was 0.637, 0.613, 0.605 and 0.660, respectively, when NLR, CAR, CRP/L, and NLPR were evaluated for mortality. According to these results, NLPR had the highest sensitivity (cut-off >0.026, 64% in ICU admission, 63% in mortality) and CRP/L had the highest specificity (cut-off >60, 56% in ICU admission, 63% in mortality).

Similarly, SII was higher in patients who admitted to ICU compared to those who did not in the study by Nalbant et al. (20). In ROC analysis, when SII was ≥813.6, AUC was 0.689, sensitivity was 70.8% and specificity was 66% (20). NLR and CAR were higher in the mortality group than in the survival group in the study by Acehan et al. (21). CAR was an independent risk factor for mortality. AISI, NLPR, SII, and SIRI were associated with admission to ICU in the study by Hamad et al. (22). Similar to this study, NLRP sensitivity was the highest index (cut-off >0.0195, 61.3%) (22).

Acar et al. (23) studied LCRP, which is the opposite of CRP/L, and it was associated with mortality in patients with COVID-19 (cut-off <1, AUC: 0.817, sensitivity 100%, specificity 86.8%). In this study, procalcitonin levels were associated with both ICU admission and mortality. It was an independent risk factor for ICU admission (OR: 1.492, 95% CI: 1.031-2.160) in the multivariate analysis. Similar to our study, Tong-Minh et al. (24) showed correlation between procalcitonin levels and mortality and procalcitonin was an independent risk factor for mortality (OR: 2.11, 95% CI: 1.36-3.61) in multivariate analysis.

Study Limitations

The limitations of the study are that it cannot be generalized to the population without being supported by prospective studies, since it was single-centered and retrospective. Patients with malignancy and immunosuppression that may affect hematological

parameters such as lymphocytes and neutrophils were not included in the study, but other potential causes that could affect these parameters were not evaluated in this study.

Conclusion

NLR, CAR, NLPR, and CRP/L were associated with both ICU admission and mortality in patients with COVID-19. SII was associated with only admission to the ICU. In addition, it was evaluated that high procalcitonin levels may be an independent risk factor for admission to ICU, and RDW-SD may be an independent risk factor for both mortality and admission to ICU. Using algorithms that can be developed with these measured parameters, it may be possible to predict the prognosis of COVID-19 patients and to decide on anti-inflammatory treatment for the severe course of the disease.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of the Ümraniye Training and Research Hospital (date: November 19, 2020, no: 346).

Informed Consent: Informed consent was waived because of the retrospective nature of the study.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: K.C., T.Ç., E.S., Ö.A., Concept: K.C., T.Ç., E.S., Ö.A., Design: K.C., T.Ç., E.S., Ö.A., Data Collection or Processing: K.C., T.Ç., E.S., Ö.A., Analysis or Interpretation: K.C., T.Ç., E.S., Ö.A., Literature Search: K.C., T.Ç., E.S., Ö.A., Writing: K.C., T.Ç., E.S., Ö.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. World Health Organization. WHO Coronavirus (COVID-19) Dashboard. (cited 2022 January 09). Available from: https://covid19.who.int/
2. Elavarasi A, Raju Sagiraju HK, Garg RK, Ratre B, Sirohiya P, Gupta N, et al. Clinical features, demography, and predictors of outcomes of SARS-CoV-2 infection at a tertiary care hospital in India: A cohort study. Lung India. 2022;39:16-26. [Crossref]
3. Bordallo B, Bellas M, Cortez AF, Vieira M, Pinheiro M. Severe COVID-19: what have we learned with the immunopathogenesis? Adv Rheumatol. 2020;60:50. [Crossref]
4. Huang Z, Fu Z, Huang W, Huang K. Prognostic value of neutrophil-to-lymphocyte ratio in sepsis: A meta-analysis. Am J Emerg Med. 2020;38:641-647. [Crossref]
5. Barale C, Melchionda E, Morotti A, Russo I. Prothrombotic Phenotype in COVID-19: Focus on Platelets. Int J Mol Sci. 2021;22:13638. [Crossref]
6. Sanghera C, Teh JJ, Pinato DJ. The systemic inflammatory response as a source of biomarkers and therapeutic targets in hepatocellular carcinoma. Liver Int. 2019;39:2008-2023. [Crossref]
7. Jin M, Yuan S, Yuan Y, Yi L. Prognostic and Clinicopathological Significance of the Systemic Immune-Inflammation Index in Patients With Renal Cell Carcinoma: A Meta-Analysis. Front Oncol 2021;11:735803. [Crossref]
8. Wu Y, Li C, Zhao J, Yang L, Liu F, Zheng H, et al. Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios predict chemotherapy outcomes and prognosis in patients with colorectal cancer and synchronous liver metastasis. World J Surg Oncol. 2016;14:289. [Crossref]
9. Eissa M, Shaarawy S, Abdellateif MS. The Role of Different Inflammatory Indices in the Diagnosis of COVID-19. Int J Gen Med. 2021;14:7843-7853. [Crossref]
10. Fois AG, Paliogiannis P, Scano V, Cau S, Babudieri S, Perra R, et al. The Systemic Inflammation Index on Admission Predicts In-Hospital Mortality in COVID-19 Patients. Molecules. 2020;25:5725. [Crossref]
11. World Health Organization. Therapeutics and COVID-19. Living guideline, 2021 Sep 24. (cited 2022 January 01). Available from: https://apps.who.int/iris/bitstream/handle/10665/345356/WHO-2019-nCoV-therapeutics-2021.3-eng.pdf?sequence=1&isAllowed=y
12. Republic of Turkey Ministry of Health. Guide of COVID-19 (SARS-Cov-2 infection). Updated on Dec 20, 2021. (cited 2022 January 01). Available from: https://covid19.saglik.gov.tr/Eklenti /42169/0/covid-19rehberieriskinhastayonetimive tedavi -20122021v6pdf.pdf –
13. La Torre G, Marte M, Massetti AP, Carli SM, Romano F, Mastroianni CM, et al. The neutrophil/lymphocyte ratio as a prognostic factor in COVID-19 patients: a case-control study. Eur Rev Med Pharmacol Sci. 2022;26:1056-1064. [Crossref]
14. Sun P, Qie S, Liu Z, Ren J, Li K, Xi J. Clinical characteristics of hospitalized patients with SARS-CoV-2 infection: A single arm meta-analysis. J Med Virol. 2020;92:612-617. [Crossref]
15. Taylor EH, Marson EJ, Elhadi M, Macleod KDM, Yu YC, Davids R, et al. Factors associated with mortality in patients with COVID-19 admitted to intensive care: a systematic review and meta-analysis. Anaesthesia. 2021;76:1224-1232. [Crossref]
16. Meng Y, Wang J, Wen K, Da W, Yang K, Zhou S, et al. Clinical Features and Laboratory Examination to Identify Severe Patients with COVID-19: A Systematic Review and Meta-Analysis. Biomed Res Int. 2021;2021:6671291. [Crossref]
17. Russo A, Tellone E, Barreca D, Ficarra S, Laganà G. Implication of COVID-19 on Erythrocytes Functionality: Red Blood Cell Biochemical Implications and Morpho-Functional Aspects. Int J Mol Sci. 2022;23:2171. [Crossref]
18. Pluta MP, Zachura MN, Winiarska K, Kalemba A, Kapłan C, Szczepańska AJ, et al. Usefulness of Selected Peripheral Blood Counts in Predicting Death in Patients with Severe and Critical COVID-19. J Clin Med. 2022;11:1011. [Crossref]
19. Wu Y, Li H, Zhang Z, Liang W, Zhang T, Tong Z, et al. Risk factors for mortality of coronavirus disease 2019 (COVID-19) patients during the early outbreak of COVID-19: a systematic review and meta-analysis. Ann Palliat Med. 2021;10:5069-5083. [Crossref]
20. Nalbant A, Demirci T, Kaya T, Aydın A, Altındiş M, Güçlü E. Can prognostic nutritional index and systemic immune-inflammatory index predict disease severity in COVID-19? Int J Clin Pract. 2021;75:e14544. [Crossref]
21. Acehan S, Gulen M, Isikber C, Kaya A, Unlu N, Ince C, et al. C-reactive protein to albumin ratio is associated with increased risk of mortality in COVID-19 pneumonia patients. Cukurova Med J. 2021;46:1449-1458. [Crossref]
22. Hamad DA, Aly MM, Abdelhameid MA, Ahmed SA, Shaltout AS, Abdel-Moniem AE, et al. Combined Blood Indexes of Systemic Inflammation as a Mirror to Admission to Intensive Care Unit in COVID-19 Patients: A Multicentric Study. J Epidemiol Glob Health. 2021;12:64-73. [Crossref]
23. Acar E, Demir A, Yıldırım B, Kaya MG, Gökçek K. The role of hemogram parameters and C-reactive protein in predicting mortality in COVID-19 infection. Int J Clin Pract. 2022;75:e14256. [Crossref]
24. Tong-Minh K, van der Does Y, Engelen S, de Jong E, Ramakers C, Gommers D, et al. High procalcitonin levels associated with increased intensive care unit admission and mortality in patients with a COVID-19 infection in the emergency department. BMC Infect Dis. 2022;22:165. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2022.29494

Hamidiye Med J 2022;3(2):140-146

A Meta-analysis of the Hospital Stay and Hypoxic Effect of Neuromuscular Blocking Agent Antagonists, Sugammadex and Neostigmine

Nöromusküler Bloke Edici Ajan Antagonistleri olan Sugammadeks ve Neostigmin'in Hastanede Kalış ve Hipoksik Etkisi Üzerine bir Meta-analizi

Habip Yılmaz[1], Fatih Özçelik[2]

[1]*İstanbul Health Directorate, Department of Anesthesiology and Reanimation, Public Hospitals Services Presidency-1, İstanbul, Türkiye*

[2]*University of Health Sciences Türkiye Hamidiye Faculty of Medicine, Department of Medical Biochemistry, İstanbul, Türkiye*

ABSTRACT

Background: Considering that the postoperative residual curarization rate may vary between 5% and 85% depending on the anesthetic applications, according to current scientific publications, many patients recovering from anesthesia are at serious risk. Our aim in this meta-analysis study is to reveal the comparative effects of sugammadex and neostigmine drugs used for decurarization on hospitalization and hypoxia.

Materials and Methods: The terms "sugammadex", "neostigmine", "anesthesia", "neuromuscular blockade", "neuromuscular blocking agents" "sugammadex and neostigmine" were searched in the electronic databases of PubMed, DynaMed, Google Scholar. "Clinical research" as search filters, the terms "controlled clinical trial" and "randomized controlled trial" were used, and the data were analyzed by a fixed effect (I^2<25%) or random effects (I^2>25%) model according to the presence of heterogeneity.

Results: After the database search, a total of 1902 articles were found. After excluding repetitive articles, 1033 articles were reviewed. Whether they were related to the subject or not was determined by reviewing the title and summary sections. The full text of 50 articles that might be relevant is reviewed. As a result, 13 articles were included in the meta-analysis. As a result of the analysis, it was observed that the studies were heterogeneous (I^2=97.9%; I^2=90.5%). Analysis according to the random-effects model. It was found that the duration of hospital stay and SPO_2 levels after surgery were not different in patients given sugammadex and neostigmine [standardised mean difference (SMD)=-0.0042; 95% confidence interval (CI) (-0.0459-0.0375), p=0.8438; SMD=-0.0017; 95% CI (-0.01076-0.1111); p=0.9753].

Conclusion: The results of this meta-analysis show that sugammadex is no more effective in recovery from neuromuscular blockade than neostigmine in terms of hospital stay and SPO_2.

Keywords: Sugammadex, neostigmine, neuromuscular blockade, decurarization, meta-analysis

ÖZ

Amaç: Günümüzdeki bilimsel yayınlara göre post operatif rezidüel kürarizasyon oranı anestezik uygulamalara bağlı olarak %5 ile %85 arasında değişebileceği düşünülecek olursa, anesteziden uyanmakta olan birçok hasta ciddi risk altındadır. Bu meta-analiz çalışmasında amacımız dekürarizasyon için kullanılan sugammadeks ve neostigmin ilaçlarının karşılaştırmalı olarak hastane yatışı ve hipoksiye etkisini ortaya koymaktır.

Gereç ve Yöntemler: "Sugammadeks", "neostigmin", "anestezi", "nöromusküler blokaj", "nöromusküler bloke edici ajanlar" "sugammadeks ve neostigmin" terimleri PubMed, DynaMed, Google Akademik elektronik veri tabanlarında arandı. Arama filtreleri olarak "klinik araştırma", "kontrollü klinik araştırma" ve "randomize kontrollü araştırma" ifadeleri kullanıldı. Veriler heterojenite varlığına göre sabit etki (I^2<%25) ya da rastgele etki (I^2>%25) modeli ile analiz edildi.

***One of the authors of this article (FÖ) is a member of the Editorial Board of this journal. He was completely blinded to the peer review process of the article.**

Address for Correspondence: Fatih Özçelik, University of Health Sciences Türkiye Hamidiye Faculty of Medicine, Department of Medical Biochemistry, İstanbul, Türkiye
Phone: +90 532 327 99 63 E-mail: 68ozcelik@gmail.com **ORCID ID:** orcid.org/0000-0003-2439-3964

Received: 14.06.2022 **Accepted:** 17.06.2022

©Copyright 2022 by University of Health Sciences Türkiye Hamidiye Faculty of Medicine. / Hamidiye Medical Journal published by Galenos Yayınevi.

ÖZ

Bulgular: Veritabanı araştırması sonrasında toplam 1902 makaleye ulaşıldı. Tekrarlayan makaleler dışlandıktan sonra 1033 makale incelendi. Konu ile ilişkili olup olmadıkları başlık ve özet bölümlerinin gözden geçirilmesi ile tespit edildi. İlişkili olabilecek 50 makalenin tam metinleri incelendi. Sonuçta 13 makale meta-analize dahil edildi. Yapılan analiz sonucunda araştırmaların heterojen olduğu gözlendi (I^2=%97,9; I^2=%90,5). Rastgele etki modeline göre yapılan analiz sugammadeks ve neostigmin verilen hastalarda, cerrahiden sonra hastane yatış gün süresi ve SPO_2 düzeylerinin farklı olmadığını saptandı [SMD=-0,0042; %95 güven aralığı (GA) (-0,0459-0,0375), p=0,8438; SMD=-0,0017; %95 GA (-0,01076-0,1111); p=0,9753].

Sonuç: Bu meta-analizin sonuçları, sugammadeksin nöromusküler blokajı tersine çevirmede neostigminden hastanın hastanede yatış süresi ve SPO_2 açısından daha etkili olmadığını göstermektedir.

Anahtar Kelimeler: Sugammadeks, neostigmin, nöromusküler blokaj, dekürarizasyon, meta-analiz

Introduction

The inability to completely remove the neuromuscular blockade (NMB) formed during anesthesia, known as postoperative residual curarization (PORC), is important for patient morbidity and mortality. Analgesia, on the other hand, is one of the basic procedures applied in anesthesia management to provide amnesia, to get rid of the fear caused by the surgical procedure, and to provide adequate muscle relaxation. Muscle relaxants used in anesthesia applications act on the neuromuscular junction, facilitating intubation and optimizing surgical conditions (1,2,3). Various agents are also used to terminate anesthesia and reverse the effects of muscle relaxants after the surgical procedure. Sugammadex has been added to these recently. There are many recommendations in the literature regarding the use of these agents that reverse the anesthesia process. It has been published that the effects of neostigmine should be examined especially in terms of timing and spontaneous recovery, time to reach the peak, and the American Society of Anesthesiologists physicalstatus classification system (1,4,5).

Scales such as the Glasgow Coma scale and/or Aldrete score are commonly used to evaluate recovery from anesthesia (6,7). Neuromuscular monitoring during and after the operation should be essential for optimal management of neuromuscular blocking drugs. Although there are many different methods, this monitoring can be done with sustained head lift, normal pattern of respiration, sustainedhand grip, normal vital capacity and oxygen saturation, eye opening, tongue protrusion and depressor test and/or quadruple train ratio using acceleromyography principle (8,9). However, the evidence confirming the reliability of clinical signs in evaluating the adequacy of reversal from NMB is insufficient and there is no consensus on this issue (7,10).

The most commonly used decurarizing agents for reversal of NMB in anesthesia applications are sugammadex and neostigmine. Although it has been reported in many studies that sugammadex can provide faster and full-term muscle strength recovery (11,12,13), more definitive results are still needed in this regard.

For this reason, it was planned to conduct a meta-analysis including a large literature search that could reveal the difference between the decurarization effects of sugammadex and neostigmine in terms of hospital stay and SPO_2 levels.

Material and Methods

Clinical and observational studies comparing sugammadex and neostigmine for recovery from NMB caused by aminosteroid NMB agents in patients under general anesthesia were considered. Comparison of sugammadex and neostigmine used for reversal of rocuronium or vecuronium-induced NMB, English and/or Turkish article, adult patients (≥18 years old), completeness and compatibility of data, accessibility of full-text version of article and publication in a peer-reviewed journal were used as inclusion criteria for this quantitative meta-analysis. Observational studies, non-clinical studies, pediatric studies, animal experiments, lack of available data, and lack of a full-text version of the article were determined as exclusion criteria.

Articles published in PubMed, DynaMed, Google Scholar electronic databases from January 01, 2015 to April 30, 2022 were searched. Databases were searched using the terms "sugammadex", "neostigmine", "anesthesia", "neuromuscular blocking", "neuromuscular blocking agents" and "sugammadex and neostigmine". The terms "clinical trial", "controlled clinical trial" and "randomized controlled trial" were used as search filters.

Titles and abstracts of articles found in accordance with the rules set for search were independently scanned and irrelevant articles were excluded. The remaining full texts were evaluated whether they met the inclusion criteria. After the data obtained from the studies were written on the designed data collection forms, the findings were independently cross-checked by both authors. Meta-analysis of the data was performed using the PRISMA methodology.

Since the study is a meta-analysis study, it is not necessary to obtain informed consent from the patients. For this study, the necessary permission (İstanbul Provincial Health Directorate number: E-15086342-903.07.02) was obtained from the institution.

Statistical Analysis

The study was carried out using the meta-analysis technique. Heterogeneity between studies was measured using the I^2 statistic. The Cochran's Q value of 0.1 was used as the threshold to determine whether heterogeneity was present. The I^2 value of 0.05 was considered significant. Egger's regression test was used to assess the risk of publication bias. All p-values were considered 2-tailed and statistical significance 0.05. Calculations were made with R studio (version 4.1.3-2022.02.1 for Windows).

Results

After the database review, a total of 1902 articles were found. After excluding repetitive articles, 1033 articles were reviewed. Whether the articles were related to the subject or not was determined by reviewing the title and abstract sections. The full text of 50 articles that might be relevant is reviewed. As a result, 13 articles were included in this meta-analysis. The selection protocol of the study is given in Figure 1 with the PRISMA flowchart.

When 7 different articles were evaluated, which were compatible with each other, it was found that there was no difference in terms of hospital stay time in patients who were given sugammadex and neostigmine [SMD=-0.0042; 95% confidence interval (CI) (-0.0459-0.0375); p=0.8438). Q 0.0001, I^2=97.9 H=6.89, 95% CI (5.73-8.28) (Figure 2).

Eight articles that were consistent with hospital SPO_2 were evaluated. It was found that there was no difference between the patients given sugammadex and neostigmine in terms of SPO_2 [SMD=-0.0017; 95% CI (-0.01076-0.1111); p=0.9753 Q 0.0001; I^2=90.5 H=3.25 95% CI (2.48-4.26)] (Figure 3).

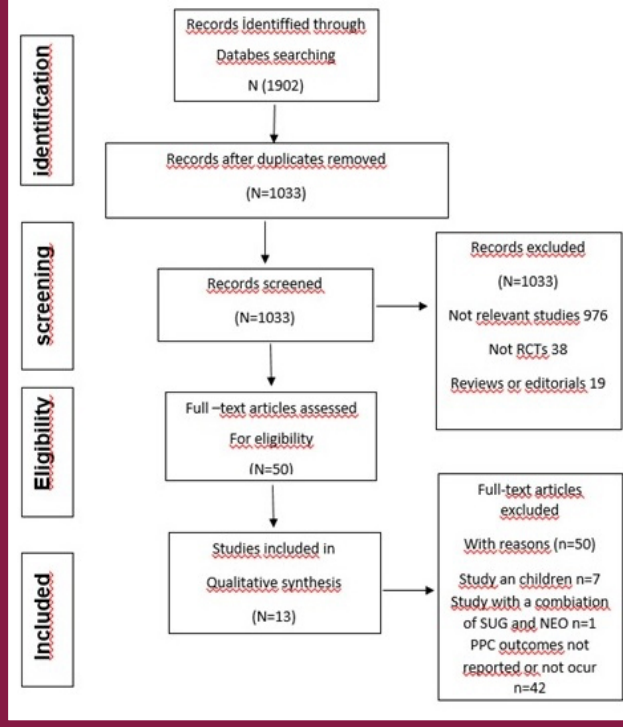

Figure 1. PRISMA flow diagram

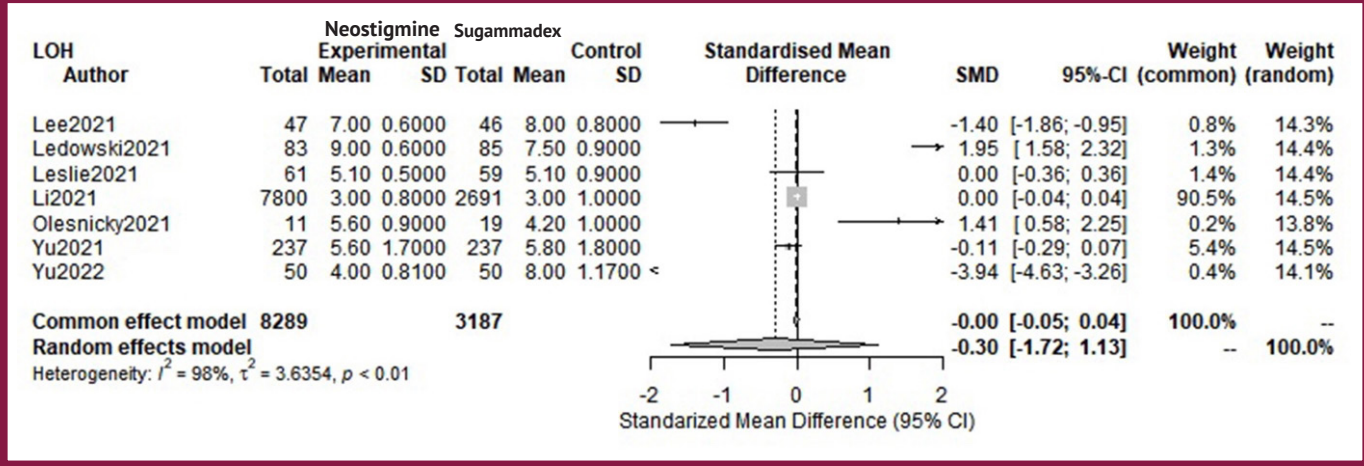

Figure 2. Forest chart related to hospital stay
CI: Confidence interval, SD: Standard deviation, SMD: Standardised mean difference

As a result of the examination for heterogeneity with Funnel plot and Egger's regression test, it was found that the risk of publication bias was low [p(LOH)=0.78; p(SPO_2)=0.96] (Figure 4).

Discussion

Despite all the developments in the field of anesthesia, PORC, which still increases the risk of mortality due to the presence of blocked nicotinic receptors in post-operative patients, has not been completely prevented. It has even been reported that 60-70% of nicotinic receptors can remain curarized without causing any clinical symptoms. The high persistence of residual NMB after surgery may cause respiratory distress and hypoxia due to any residual weakness in the jaw and tongue. It is also associated with adverse patient outcomes such as the inability to clear secretions due to lack of coordinated muscle activity of the pharynx/esophagus and the risk of aspiration. For these reasons, it is very important to detect the persistence of residual NMB. Intraoperative management of NMB is possible using peripheral nerve stimulators and subjective tactile or visual evaluation. Quantitative monitoring is also required to identify patients who have adequate reversal, who recover spontaneously, and who do not require decurarizing agents. Therefore, delayed awakening from

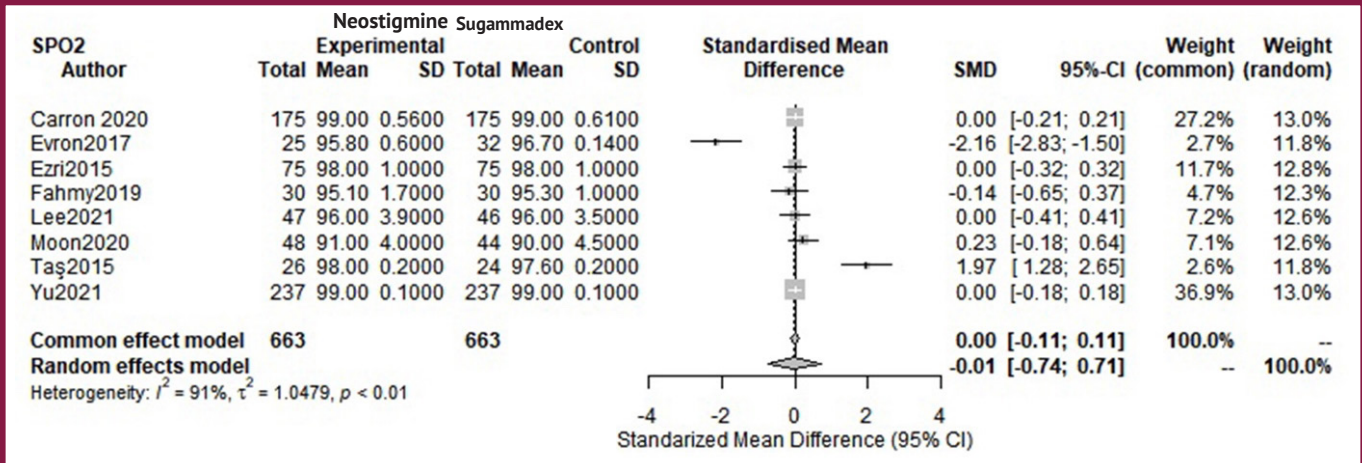

Figure 3. Forest chart for SPO_2
CI: Confidence interval, SD: Standard deviation, SMD: Standardised mean difference

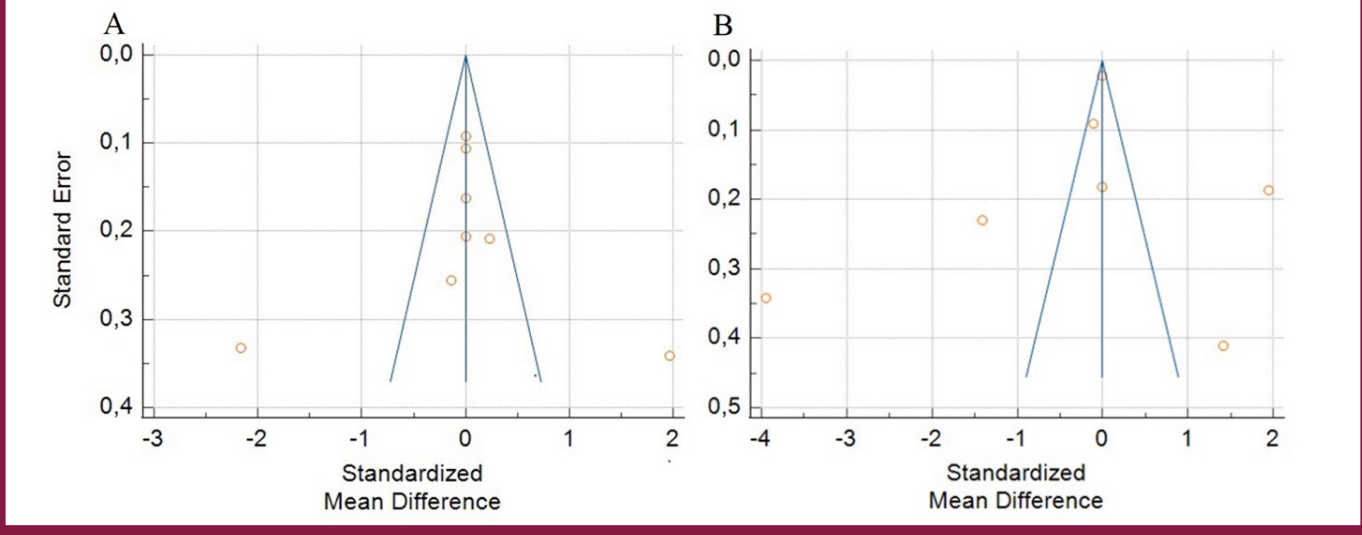

Figure 4. Funnel plots. The distribution of A) SPO_2 and B) LOH values associated with publication bias and heterogeneity is seen

anesthesia is still one of the biggest difficulties faced by the anesthesiologist (14,15,16,17). The most common cause of delayed awakening after anesthesia is anesthetic agents and drugs used in the perioperative period (18,19,20). However, some metabolic and chronic diseases (such as hypoglycemia/hyperglycemia, electrolyte imbalance, hypoxia, hypercapnia, central anticholinergic syndrome, chronic hypertension, liver disease, renal diseases, hypoalbuminemia, uremia and severe hypothyroidism), gender, obesity, cachexia, hypothermia, age and structural disorders of the central nervous system and psychological diseases may all cause delayed awakening after general anesthesia (17,18,21,22,23,24,25).

According to current scientific publications, there is limited data on the rate of PORC. It has been reported that this rate can vary between 5% and 85% depending on various anesthetic applications, and the negative effects of PORC can be seen in approximately half of the patients even with neostigmine (26). On the other hand, it has been suggested that sugammadex is more suitable for preventing the formation of residual curarization and postoperative respiratory complications. It has also been suggested that sugammadex is more suitable than neostigmine for restoring diaphragmatic function. However, there are also studies reporting that there is no difference in general between the two decurarization agents (27,28,29,30). According to the results of this meta-analysis, the fact that there was no significant difference between sugammadex and neostigmine in terms of hospital stay time, which is a measure of patient mobilization, is a results against the above information about sugammadex. However, it should be kept in mind that the study carried out is only about the length of hospital stay and SPO_2 levels, and therefore more and more comprehensive studies are needed for general judgment.

Inadequate neuromuscular monitoring and insufficient decurarization can be listed among the reasons that increase the risk of PORC. Sugammadex, a new molecule in decurarization, is a cyclodextrin group drug that selectively binds to aminosteroid rocuronium and vecuronium (NMB agents), thus providing rapid excretion and decurarization. Sugammadex has created a new option for reversing NMB and preventing residual paralysis. It shows its effect by encapsulating the free molecule very tightly at a ratio of 1:1 and forming complexes to form a stable complex. It also acts on neuromuscular blocking agents with similar aminosteroid structures such as vecuronium. Compared with neostigmine used to reverse NMB, sugammadex has been reported to be faster in reversing rocuronium-induced blockade, and patients can potentially be discharged faster after general anesthesia (31,32,33,34,35,36).

Neostigmine, a cholinesterase inhibitor, is traditionally used for decurarization. Neostigmine indirectly inactivates the enzyme by covalently binding to the acetyl cholinesterase enzyme located at the neuromuscular junction. Thus, acetylcholine cannot be broken down and competes with NMB agents for postsynaptic receptors. Neostigmine has a ceiling effect and may not generate adequate rebound at a deep NMB. In contrast to sugammadex, side effects such as bradycardia, autonomic disorders, nausea and vomiting have been reported. In addition, cholinesterase inhibitor agents used in decurarization may have serious side effects, especially since they stimulate the muscarinic system as well as nicotinic receptors. All these negative effects also increase the postoperative pulmonary complications that affect the respiratory system after anesthesia. It was found that the SPO_2 values measured during extubation of sugammadex, which is known to reverse rocuronium (or vecuronium)-induced NMB more rapidly, were not different from neostigmine in recovery from medium and deep NMB (37,38,39,40). In this meta-analysis study, which we conducted in the light of the above information, the hypoxic effects of sugammadex and neostigmine, which can be defined by SPO_2 levels, which can vary due to their autonomic effects, were compared. No difference was found in this meta-analysis in terms of hypoxic effects of both agents. Although they have different mechanisms of action on NMB, these findings show that similar SPO_2 values will be achieved with the use of sugammadex and neostigmine. Contrary to all these data, in a cohort from the USA (41), it was reported that the use of sugammadex was associated with a lower incidence of major pulmonary complications, although the exact mechanism is not known. All these studies show that there is a need for more comprehensive studies comparing the use of sugammadex and neostigmine in decurarization.

Conclusion

The results of this meta-analysis show that sugammadex is no more effective in recovery from NMB than neostigmine in terms of hospital stay and SPO_2.

Acknowledgement

I would like to thank Özlem Köksal (İstanbul Health Directorate, Public Hospitals Services Presidency-1) for her effort in preparing the meta-analysis statistics of the article.

Ethics

Ethics Committee Approval: The study is a meta-analysis study, it is not necessary.

Informed Consent: Since the study is a meta-analysis study, it is not necessary to obtain informed consent from the patients. For this study, the necessary permission (İstanbul Provincial Health Directorate number: E-15086342-903.07.02) was obtained from the institution.

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.Y., Concept: H.Y., F.Ö., Design: H.Y., F.Ö., Data Collection or Processing: H.Y., F.Ö., Analysis or Interpretation: F.Ö., Literature Search: H.Y., Writing: H.Y., F.Ö.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Raval AD, Anupindi VR, Ferrufino CP, Arper DL, Bash LD, Brull SJ. Epidemiology and outcomes of residual neuromuscular blockade: A systematic review of observational studies. J Clin Anesth. 2020;66:109962. [Crossref]
2. Mencke T, Echternach M, Kleinschmidt S, Lux P, Barth V, Plinkert PK, et al. Laryngeal morbidity and quality of tracheal intubation: a randomized controlled trial. Anesthesiology. 2003;98:1049-1056. [Crossref]
3. Martini CH, Boon M, Bevers RF, Aarts LP, Dahan A. Evaluation of surgical conditions during laparoscopic surgery in patients with moderate vs deep neuromuscular block. Br J Anaesth. 2014;112:498-505. [Crossref]
4. Martini CH, Honing GHM, Bash LD, Olofsen E, Niesters M, van Velzen M, et al. The Use of Muscle Relaxants and Reversal Agents in a Setting Without Cost Restrictions: Experience from a Tertiary Academic Hospital in the Netherlands. Ther Clin Risk Manag. 2022;18:379-390. [Crossref]
5. Plaud B, Baillard C, Bourgain JL, Bouroche G, Desplanque L, Devys JM, et al. Guidelines on muscle relaxants and reversal in anaesthesia. Anaesth Crit Care Pain Med. 2020;39:125-142. [Crossref]
6. Yhim HB, Jang YE, Lee JH, Kim EH, Kim JT, Kim HS. Comparison of the TOFscan and the TOF-Watch SX during pediatric neuromuscular function recovery: a prospective observational study. Perioper Med (Lond). 2021;10:45. [Crossref]
7. Grayling M, Sweeney BP. Recovery from neuromuscular blockade: a survey of practice. Anaesthesia. 2007;62:806-809. [Crossref]
8. Fischer J, Mathieson C. The history of the Glasgow Coma Scale: implications for practice. Crit Care Nurs Q. 2001;23:52-58. [Crossref]
9. Aldrete JA. The post-anesthetic recovery score revisited. J Clin Anesth. 1995;7:89-91. [Crossref]
10. Debaene B, Plaud B, Dilly MP, Donati F. Residual paralysis in the PACU after a single intubating dose of nondepolarizing muscle relaxant with an intermediate duration of action. Anesthesiology. 2003;98:1042-1048. [Crossref]
11. Hristovska AM, Duch P, Allingstrup M, Afshari A. The comparative efficacy and safety of sugammadex and neostigmine in reversing neuromuscular blockade in adults. A Cochrane systematic review with meta-analysis and trial sequential analysis. Anaesthesia. 2018;73:631-641. [Crossref]
12. Togioka BM, Yanez D, Aziz MF, Higgins JR, Tekkali P, Treggiari MM. Randomised controlled trial of sugammadex or neostigmine for reversal of neuromuscular block on the incidence of pulmonary complications in older adults undergoing prolonged surgery. Br J Anaesth. 2020;124:553-561. [Crossref]
13. Della Rocca G, Pompei L, Pagan DE Paganis C, Tesoro S, Mendola C, Boninsegni P, et al. Reversal of rocuronium induced neuromuscular block with sugammadex or neostigmine: a large observational study. Acta Anaesthesiol Scand. 2013;57:1138-1145. [Crossref]
14. Fawcett WJ, Dash A, Francis GA, Liban JB, Cashman JN. Recovery from neuromuscular blockade: residual curarisation following atracurium or vecuronium by bolus dosing or infusions. Acta Anaesthesiologica Scand. 1995;39:288-293. [Crossref]
15. Eriksson LI, Sundman E, Olsson R, Nilsson L, Witt H, Ekberg O, et al. Functional assessment of the pharynx at rest and during swallowing in partially paralyzed humans: Simultaneous videomanometry and mechanomyography of awake human volunteers. Anesthesiology. 1997;87:1035-1043. [Crossref]
16. Thilen SR, Weigel WA. Neuromuscular Blockade Monitoring. Anesthesiol Clin. 2021;39:457-476. [Crossref]
17. Misal US, Joshi SA, Shaikh MM. Delayed recovery from anesthesia: A postgraduate educational review. Anesth Essays Res. 2016;10:164-172. [Crossref]
18. Sinclair R, Faleiro RJ. Delayed recovery of consciousness after anaesthesia. Contin Educ Anaesth Crit Care Pain. 2006;6:124-128. [Crossref]
19. Miller RD, editor. Miller's Anesthesia. 7th ed. United States of America: Elsevier, Churchill; 2010;2722-2723. [Crossref]
20. Sarangi S. Delayed awakening from anaesthesia. Internet J Anesthesiol. 2008;19:1-4. [Crossref]
21. Apfelbaum JL, Grasela TH, Hug CC Jr, McLeskey CH, Nahrwold ML, Roizen MF, et al. The initial clinical experience of 1819 physicians in maintaining anesthesia with propofol: Characteristics associated with prolonged time to awakening. Anesth Analg. 1993;77(Suppl 4):S10-S14. [Crossref]
22. Buchanan FF, Myles PS, Leslie K, Forbes A, Cicuttini F. Gender and recovery after general anesthesia combined with neuromuscular blocking drugs. Anesth Analg. 2006;102:291-297. [Crossref]
23. Tsai HJ, Chen CC, Chang KY. Patients and surgery-related factors that affect time to recovery of consciousness in adult patients undergoing elective cardiac surgery. J Chin Med Assoc. 2011;74:345-349. [Crossref]
24. Shaikh SI, Lakshmi RR. Delayed awakening after anaesthesia – A challenge for an anaesthesiologist. Int J Biomed Adv Res. 2014;5:352-354. [Crossref]
25. Frost EA. Differential diagnosis of delayed awakening from general anesthesia: A review. Middle East J Anaesthesiol. 2014;22:537-548. [Crossref]
26. Hayes AH, Mirakhur RK, Breslin DS, Reid JE. McCourt KC. Postoperative residual block after intermediate-acting neuromuscular blocking drugs. Anaesthesia. 2001;56:312-318. [Crossref]
27. Suzuki T, Masaki G, Ogawa S. Neostigmine-induced reversal of vecuronium in normal weight, overweight and obese female patients. Br J Anaesth. 2006;97:160-163. [Crossref]
28. Gaszynski T, Szewczyk T, Gaszynski W. Randomized comparison of sugammadex and neostigmine for reversal of rocuronium-induced muscle relaxation in morbidly obese undergoing general anaesthesia. Br J Anaesth. 2012;108:236-239. [Crossref]
29. Zhang YG, Chen Y, Zhang YL, Yi J. Comparison of the effects of neostigmine and sugammadex on postoperative residual curarization and postoperative pulmonary complications by means of diaphragm and lung ultrasonography: a study protocol for prospective double-blind randomized controlled trial. Trials. 2022;23:376. [Crossref]
30. Ezri T, Evron S, Petrov I, Schachter P, Berlovitz Y, Shimonov M. Residual Curarization and Postoperative Respiratory Complications Following Laparoscopic Sleeve Gastrectomy. The Effect of Reversal Agents: Sugammadex vs. Neostigmine. J Crit Care Med (Targu Mures). 2015;1:61-67. [Crossref]

31. Sayın M. Kas Gevşeticiler. Keçik Y (ed). Temel Anestezi. Ankara: Güneş Tıp Kitapevi. 2012:131-151.
32. Nag K, Singh DR, Shetti AN, Kumar H, Sivashanmugam T, Parthasarathy S. Sugammadex: A revolutionary drug in neuromuscular pharmacology. Anesth Essays Res. 2013;7:302-306.
33. Bom A, Bradley M, Cameron K, Clark JK, Van Egmond J, Feilden H, et al. A novel concept of reversing neuromuscular block: Chemical encapsulation of rocuronium bromide by a cyclodextrin-based synthetic host. Angew Chem Int Ed Engl. 2002;41:266-270.
34. Miller R. Miller's Anesthesia, Philadelphia, PA : Elsevier/Saunders; 2015:1195.
35. Epemolu O, Bom A, Hope F, Mason R. Reversal neuromuscular blockade and simultaneous increase in plasma rocuronium consantration after the intravenous infusion of the novel reversal agent Org 25969. Anesthesiology. 2003;99:632-637.
36. Flockton EA, Mastronardi P, Hunter JM, Gomar C, Mirakhur RK, Aguilera L, et al. Reversal of rocuronium-induced neuromuscular block with sugammadex is faster than reversal of cisatracurium-induced block with neostigmine. Br J Anaesth. 2008;100:622-630.
37. Büyükkoçak Ü. Antagonism of Neuromuscular Block and Sugammadex. Turkiye Klinikleri J Anest Reanim-Special Topics. 2011;4:58-66.
38. Sacan O, White PF, Tufanogullari B, Klein K. Sugammadex reversal of rocuronium-induced neuromuscular blockade: A comparison with neostigmine-glycopyrrolate and edrophonium-atropine. Anesth Analg. 2007;104:569-574. [Crossref]
39. Fiekers JF. Concentration-dependent effects of neostigmine on the endplate acetylcoline receptor channel complex. J Neurosci. 1985;5:502-514. [Crossref]
40. Li G, Freundlich RE, Gupta RK, Hayhurst CJ, Le CH, Martin BJ, et al. Postoperative Pulmonary Complications' Association with Sugammadex versus Neostigmine: A Retrospective Registry Analysis. Anesthesiology. 2021;134:862-873. [Crossref]
41. Kheterpal S, Vaughn MT, Dubovoy TZ, Shah NJ, Bash LD, Colquhoun DA, et al. Sugammadex versus neostigmine for reversal of neuromuscular blockade and postoperative pulmonary complications (STRONGER): a multicenter matched cohort analysis. Anesthesiology. 2020;132:1371-1381. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2022.25733

Hamidiye Med J 2022;3(2):147-152

Comparison of Pain Severity with VAS Score in Preoperative and Postoperative Periods After Laparoscopic Cystectomy in Isolated Endometriomas

İzole Endometriomalarda Laparoskopik Kistektomi Sonrası Preoperatif ve Postoperatif Dönemde Ağrı Şiddetinin VAS Skoru ile Karşılaştırılması

Serpil Akyüz, Esra Tamburacı, Burak Karadağ, Barış Mülayim

University of Health Sciences Türkiye, Antalya Training and Research Hospital, Clinic of Gynecology and Obstetrics, Antalya, Türkiye

ABSTRACT

Background: In this study, it was investigated whether laparoscopic surgery reduces dysmenorrhea, pelvic pain, dyspareunia and abdominal distension evaluated with visual analog scale (VAS) in the postoperative period compared to the preoperative period in patients diagnosed with isolated endometrioma.

Materials and Methods: The preoperative and postoperative VAS scores of 36 cases with isolated endometrioma were compared among 197 patients who applied with pelvic pain and underwent laparoscopic surgery with an endometrioma diagnosis between 2017 and 2020. Patients were asked to complete a questionnaire containing a 100 mm VAS scale that included the four components of pre- and post-operative endometriosis-related pain (dysmenorrhea, pelvic pain, dyspareunia, and abdominal distension). Patients with deep infiltrative endometriosis, peritoneal endometriosis, related severe intraoperative adhesions, those who had previously undergone endometriosis surgery, and those who had received hormonal therapy for endometriosis or endometrioma before the surgery were found to be excluded from the study.

Results: The VAS scores of the patients for cyclic-non-cyclic pelvic pain, dysmenorrhea, dyspareunia, and abdominal distension decreased significantly in the postoperative period compared to the preoperative period ($p<0.05$).

Conclusion: This study determined that the symptoms of cyclic-non-cyclic pelvic pain, dysmenorrhea, dyspareunia, and abdominal distension after laparoscopic surgery in patients with isolated endometrioma were significantly reduced compared to the period before surgery. In addition, CA-125 biomarker results were significantly reduced after laparoscopic surgery in patients with isolated endometrioma.

Keywords: Endometrioma, VAS score, pelvic pain, Ca125

ÖZ

Amaç: Bu çalışmada izole endometrioma tanısı konan hastalarda laparoskopik cerrahinin postoperatif dönemde görsel analog skala (VAS) ile değerlendirilerek dismenore, pelvik ağrı, disparoni ve abdominal distansiyonun preoperatif döneme göre azaltıp azaltmadığı araştırıldı.

Gereç ve Yöntemler: 2017 ve 2020 yılları arasında pelvik ağrı şikayetiyle başvuran ve endometrioma tanısı ile laparoskopik cerrahi uygulanan 197 hastanın arasından izole endometrioma tespit edilen 36 olgunun preoperatif ve postoperatif VAS skorları karşılaştırıldı. Hastalardan ameliyat öncesi ve sonrası endometriozis ile ilişkili ağrının (dismenore, pelvik ağrı, disparoni ve abdominal distansiyon) dört bileşenini içeren 100 mm'lik bir VAS ölçeğini içeren bir anketi doldurmaları istendi. Derin infiltratif endometriozis, peritoneal endometriozis ve buna bağlı ciddi intraoperatif adezyonları bulunan, daha önce endometriozis cerrahisi geçirenler, operasyondan önce endometriozis veya endometrioma nedeniyle hormonal tedavi alan hastalar çalışma dışında tutuldu.

Bulgular: Hastaların siklik-non-siklik pelvik ağrı, dismenore, disparoni ve abdominal distansiyon VAS skorlarında preoperatif döneme kıyasla postoperatif dönemde anlamlı düzeyde azalma olduğu izlendi ($p<0,05$).

Address for Correspondence: Esra Tamburacı, University of Health Sciences Türkiye, Antalya Training and Research Hospital, Clinic of Gynecology and Obstetrics, Antalya, Türkiye

Phone: +90 532 050 80 74 E-mail: dresratamburaci@gmail.com **ORCID ID:** orcid.org/0000-0002-9864-9160

Received: 16.04.2022 **Accepted:** 30.06.2022

©Copyright 2022 by University of Health Sciences Türkiye Hamidiye Faculty of Medicine. / Hamidiye Medical Journal published by Galenos Yayınevi.

ÖZ

Sonuç: Bu araştırmada izole endometrioması olan hastalarda laparoskopik cerrahi sonrası siklik-non-siklik pelvik ağrı, dismenore, disparoni ve abdominal distansiyon semptomlarının cerrahi öncesine göre belirgin azaldığı saptanmıştır. Ayrıca izole endometriomalı hastalarda laparoskopik cerrahi sonrası CA-125 biyobelirteç sonuçlarının anlamlı derecede azaldığı bulundu.

Anahtar Kelimeler: Endometrioma, VAS skoru, pelvik ağrı, Ca125

Introduction

Endometriosis is a disease characterized by the presence of the endometrial gland and stroma outside the endometrium. Its most common implantation site is the pelvic organs and peritoneum, but it can also be seen in extrapelvic areas such as the bowel, bladder, or lung. It can induce problems from minimal lesions to large adhesions that can completely distort the anatomy (1).

Endometriosis is a hormone-dependent disease and is, therefore, most common in women of reproductive age. The prevalence of surgically diagnosed endometriosis has been reported to be 10% between the ages of 15 and 49 years (2). Its prevalence in asymptomatic women ranges from 2% to 22%, while it is reported to be 21-47% in infertile women (3) and 71-87% in those with pelvic pain (2,3,4). On the other hand, the prevalence of isolated endometrioma is approximately 10-15% (4,5). It has been proven that endometrioma causes pain in these patients as well (5). Although several studies have been on endometriosis, the disease's prevalence, pathophysiology, natural history, and optimal treatment are still being investigated, and treatment methods are still being developed (1,2).

The type of endometriosis that only affects the ovaries is isolated endometriomas, which are benign, estrogen-dependent ovarian cysts. It is significant since it is a prevalent disease that also produces issues that have a detrimental impact on social life, and it has been the topic of numerous studies. The most common symptoms are dysmenorrhea, dyspareunia, non-cyclic pelvic pain, and sub-infertility.

In this study, surgical laparoscopy was performed on patients with isolated endometrioma, and it was aimed to discuss the severity of dysmenorrhea, pelvic pain, dyspareunia and abdominal distension in the preoperative and postoperative period by comparing them with the VAS method, in the light of the literature.

Material and Methods

In this prospective clinical study, 36 cases with isolated endometrioma were included among 197 patients operated on for endometrioma in University of Health Sciences Türkiye, Antalya Training and Research Hospital, Gynaecology and Obstetrics Clinic between October 2017 and October 2020. Informed consent was obtained from all patients. Before starting the study, the approval of the Local Ethics Committee of our hospital was obtained (decision number: 21/09/2017-13/7). Endometrioma was diagnosed by ultrasonography (USG), and Philips ClearVue 650 Brand USG and C9-4v Active Array transvaginal probe were used for measurements. Patients with deep infiltrative and peritoneal endometriosis, related severe intraoperative adhesions, those who had previously undergone endometriosis surgery, and those who had received hormonal therapy for endometriosis or endometrioma before the operation were excluded from the study. All operations were performed with the laparoscopic method, and the visual analogue scale (VAS) method was used to compare the severity of dysmenorrhea, pelvic pain, dyspareunia, and abdominal distension. The VAS is not a detailed assessment, and the patient is asked to rate the intensity of pain at rest or during an activity at a scale with a length of 10 cm, usually between 1-10 cm or 1-100 mm. This scale consists of a horizontal or vertical straight line. The line has a value of 0 at the beginning and a value of 10 at the end. A value of 0 means no pain, a value of 10 means unbearable pain. VAS is a widely used scale for the assessment of pain severity. The patient was asked to mark the pain she felt on this line, and the point he marked was measured in cm (6).

0 (No Pain)_____(Unbearable Pain)_10

Operation Technique

An umbilical 10 mm trocar was entered under general anaesthesia using the direct trocar insertion technique. After the pneumoperitoneum was created, three 5 mm trocars were inserted by laparoscopic observation, approximately 3 cm above the bilateral symphysis pubis, lateral to the rectus muscles, and 2 cm above the symphysis pubis, in the midline. Patients with deep infiltrative endometriosis or peritoneal endometriosis were excluded from the study. The contents of isolated endometriomas were aspirated, and the capsules were excised.

One day before the operation, the patients filled out a questionnaire evaluating pain using a 100 mm VAS including four components of endometriosis-related pain (dysmenorrhea, pelvic pain, dyspareunia, and abdominal distension). A scale (0 -100 mm) was used, where 0 represents no pain and 100 represents the worst pain imaginable (Annex-1). In addition, demographic characteristics [age, parity, body mass index (BMI)], operative characteristics,

and length of hospital stay of the patients were noted. The same symptoms were re-evaluated with 100 mm VAS in the postoperative 3rd-month follow-up of the patients and compared with their preoperative values.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) V.25 software was used in the analysis of the data. The normality of the data was checked by considering the Kolmogorov-Smirnov test and Shapiro-Wilk test. Wilcoxon Signed Ranks test and Paired samples t-test were used for repeated measurements. Normally distributed data are shown as mean ± standard deviation, and non-normally distributed data are expressed as median (minimum-maximum). The categorical data were expressed with n (number) and percentages (%). The data were analysed at a confidence interval of 95%, and a p-value of <0.05 was considered to be statistically significant.

Results

The study included 36 patients with isolated endometrioma among 197 patients who had endometriosis operation. Demographic characteristics of the patients included in this study were summarized in Table 1. The mean age of the patients included in the study is 30.8±7.1 (minimum 18, maximum 42), mean weight 64.8±13.12 kg (minimum 43 kg, maximum 100 kg), mean BMI 24.38±5.06 kg/m^2, and median gravida was 0.5 (0-5), parity was 0 (0-3). When the operation histories were examined, 3 (8.3%) patients had a laparotomic myomectomy, 7 (19.4%) patients had a caesarean section, 2 (5.6%) patients had laparoscopic cystectomy, and 1 (2.8%) had laparotomic cystectomy operations. Moreover, it was observed that 34 (94.4%) patients did not have any additional disease, 1 (2.8%) patient had mitral valve prolapse (MVP), and 1 (2.8%) patient had a familial Mediterranean fever (FMF). The clinical, laboratory, and surgical data of the patients are given in Table 2. Bilateral endometrioma was detected in 13 (36.1%) of the patients. The mean operation time was 66.75±28.14 minutes, and the mean amount of bleeding was 133.6±149.8 cc. The comparison of VAS scores, CA-125, and hemoglobin values calculated in the preoperative and postoperative period are presented in Table 3. While the pelvic pain VAS score was 65±16.47 in the preoperative period, it was 40.83±16.27 in the postoperative period (p=0.001), the dysmenorrhea VAS score was 63.61±16.58 preoperatively and 37.77±16.92 postoperatively (p=0.001), the dyspareunia VAS score was 63.88±17.93 preoperatively and 45±19.63 postoperatively (p=0.001), and the abdominal distension VAS score was 54.44±14.02 preoperatively and 36.11±18.55 postoperatively (p=0.001). Preoperative CA-125 was 76.7±85.6, postoperative 22.78±17.01, and a significant difference was found between them (p<0.05). While the hemoglobine value was 12.55±1.06 preoperatively, it was 11.01±1.06 postoperatively. Preoperative-postoperative hemoglobin difference was 1.54±0.8 (g/dL). The difference between preoperative and postoperative hemoglobin values was statistically significant (p=0.001).

Table 1. Demographic characteristics of the patients

Age (mean ± SD)		**30.8±7.1**
Height (m) (mean ± SD)		1.63±0.04
Weight (kg) (mean ± SD)		64.8±13.12
BMI (kg/m^2) (mean ± SD)		24.38±5.06
Gravida (median)		0 (0-5)
Parity (median)		0 (0-3)
Abortus (median)		0 (0-3)
D/C (median)		0 (0-2)
Ectopic pregnancy (median)		0 (0-0)
Comorbidity	No	34 (94.4%)
	MVP	1 (2.8%)
	FMF	1 (2.8%)
Operation history	No	23 (63.9%)
	Laparotomic myomectomy	3 (8.3%)
	Cesarean section	7 (19.4%)
	Laparoscopic cystectomy	2 (5.6%)
	Laparotomic cystectomy	1 (2.8%)

BMI: Body mass index, MVP: Mitral valve prolapse, FMF: Familial mediterranean fever, D/C: Dilatation and curettage, SD: Standard deviation

Discussion

Endometriosis constitutes an important part of all patients with pelvic pain syndrome. There are no definitive criteria to determine whether endometriosis lesions cause pain symptoms.

Table 2. Clinical, laboratory and surgical data of patients

Cyst location (n,%)	**Unilateral**	**23 (63. 9)**
	Bilateral	**13 (36.1)**
1st cyst	Size 1 (mm) (mean ± SD)	52.94±19.80
	Size 2 (mm) (mean ± SD)	50.27±20.16
2nd cyst	Size 1 (mm) (mean ± SD)	45.30±17.20
	Size 2 (mm) (mean ± SD)	43.69±19.19
Operation time (min.) (mean ± SD)		66.75±28.14
Amount of bleeding (cc) (median)		60 (0-600)
Preoperative-postoperative hemoglobin difference (g/dL) (mean ± SD)		1.54±0.8

SD: Standard deviation

Table 3. Comparison of patients' preoperative and postoperative VAS scores, CA125 and HB values

VAS scores	Preoperative	Postoperative	p
*Pelvic pain (mm) (median)	70 (30-90)	40 (10-70)	**<0.001**[1]
*Dysmenorrhea (mm) (median)	60 (30-90)	40 (10-70)	**<0.001**[1]
*Dyspareunia (mm) (median)	70 (30-90)	50 (10-80)	**<0.001**[1]
*Abdominal distention (mm) (median)	50 (30-80)	40 (10-60)	**<0.001**[1]
*CA 125 (U/mL) (median)	54.05 (3.2-419)	20.15 (0-62)	**0.001**[1]
*Hemoglobin (g/dL) (mean ± SD)	12.55±1.06	11.01±1.05	**<0.001**[2]

[1]Wilcoxon Signed Ranks test, [2]Paired samples t-test, SD: Standard deviation, VAS: Visual analog scale

In this study, it was observed that CA-125 biomarker results and pain symptoms decreased by 20-25% after laparoscopic surgery in patients with isolated endometrioma, and significantly improved. Although there is some evidence that isolated ovarian endometriomas also cause pain, it is important to resolve the association of endometriosis and pain, detect a condition that reports the interdependence between serum CA-125 measurement, history, physical examination data, imaging methods and symptoms, and plan their treatment accordingly (7,8).

Based on the findings of this study, the VAS scores of 36 patients with isolated endometrioma who underwent laparoscopic cystectomy and confirmed by the pathology results decreased significantly in the postoperative period compared to the preoperative period.

A systematic review reported that the VAS is the most commonly used scale and is helpful for clinicians to evaluate treatment response in endometriosis-related pain (9). The VAS rating method is favoured because it is simple, quick, and economical. The relationship between pelvic pain symptoms and endometrioma has not been clearly defined (10). Ballard et al. (11) investigated whether different dimensions of chronic pelvic pain before laparoscopy are beneficial in the diagnosis of endometriosis. They conducted a questionnaire to assess the definitions, areas, and intensity of pain and observed differences in pain dimensions between women with and without endometriosis and those with deep and superficial endometriosis (11).

According to the ASRM's recommendation in 2014, laparoscopic treatment of endometriosis provides relief in pain and therefore treats endometriotic lesions seen during a diagnostic laparoscopy (12).

According to a recent Cochrane meta-analysis and the results of 5 randomized controlled studies, it was determined that the pain complaints of endometriosis patients treated with laparoscopic surgery were significantly reduced compared to patients who underwent diagnostic laparoscopy but were not treated (13). Improvement in pain score may be a response to surgery-induced regression of neurogenic inflammation of the pelvic organs, hyperalgesia, and dysreflexia (14).

In another study, the efficacy of laparoscopic excision in reducing pain in patients with visually diagnosed peritoneal endometriosis (without any signs of deep endometriosis) in the treatment of chronic pelvic pain was investigated (confirmed by pathology result that the excised tissues are endometriosis), and it was observed that the pain decreased in the postoperative period with a mean follow-up of 13 months (15). Similar to these results, in our study it was determined that the pain findings of the patients who presented with pain and were diagnosed with isolated endometrioma improved by 20-25% as a result of the treatment. The number of patients with only ovarian endometrioma seems to be very low. This demonstrates that our research is unique and that no other study of its kind has ever been conducted. There is a common opinion in the literature that pain related to endometriosis is mainly caused by deep and peritoneal endometriosis (12). However, nerve fibres in ovarian endometriomas were generally seen in the endometriotic stroma and around the endometriotic glands, as in peritoneal endometriosis and deep infiltrative endometriosis (16,17).

Tokushige et al. (17) also presented data to support this study and confirmed the presence of nerve fibres in ovarian endometriomas by detecting neuronal markers such as substance P, neuropeptide Y, and vasoactive intestinal peptide. In another study, Odagiri et al. (18) showed that ovarian endometriomas have more intense staining than peritoneal endometriosis by using neuron cell adhesion molecule staining to prove neuronal fibres. As a result of our study, we found similar findings with these two studies.

CA-125 is the most studied and identified marker, whose increase has been reported in endometriosis patients (14,19,20). In the study of Hirsch et al. (21), it was mentioned that CA-125 could describe pelvic pain and be a predictor for the presence of endometriosis in symptomatic patients. We also examined CA-125 in our study and found that its postoperative values decreased significantly. Although a

Cochrane meta-analysis by Nisenblat et al. (22) showed that serum CA-125 is not helpful as a diagnostic tool for endometriosis, it has been noted that it may have a potential use if post-surgical monitoring is required. When we evaluate the decrease in CA-125 values along with the decrease in VAS scores after laparoscopic cystectomy, we think that the patient benefited from the treatment and that CA-125 can be used in the follow-up of treatment, although it is not very helpful in diagnosis.

In general, the inhomogeneity of the methods employed to associate VAS ratings and endometriosis pictures in the literature made comparing our results challenging. These may include the methodology of the studies, the surgical method performed, or the post-surgical period in which VAS scores were collected, and the shape, location, or even definition of pain.

As a result, it can be concluded that there is a statistically significant improvement in short or long-term follow-ups in the majority of studies that evaluate pain using VAS scoring (11,14). In fact, the pain was also reduced significantly in VAS score evaluation studies in which 2 or 5 years of follow-up were published (14,23).

Study Limitations

Our research has some limitations. Despite the improvement in symptoms in VAS scores and CA-125 biomarker according to our results, we believe that studies with longer-term follow-up with more patients are necessary due to the subjective nature of the scale we used in our study and the limitations of our patient number and follow-up period. Furthermore, it is necessary to conduct long-term controlled studies in which pain detection, classification, and operational benefit can be evaluated with more detailed questionnaires such as severity, location, and type of pain developed for VAS scoring based only on patient selection.

Conclusion

It was observed that CA-125 biomarker results and pain symptoms decreased by 20-25% after laparoscopic surgery in patients with isolated endometrioma. We can conclude that pelvic pain was also reduced in these cases after laparoscopic surgery.

Ethics

Ethics Committee Approval: We adhered to the Declaration of Helsinki principles. Ethical approval was attained from the University of Health Sciences Türkiye, Antalya Training and Research Hospital, Local Clinical Research Ethics Committee (date and decision number: 21/09/2017:13/07).

Informed Consent: Written informed consent to participate and publish was obtained from all individual participants included in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.A., B.K., Concept: S.A., E.T., B.M., Design: S.A., E.T., B.K., Data Collection or Processing: S.A., B.K., B.M., Analysis or Interpretation: S.A., E.T., B.M., Literature Search: S.A., E.T., B.K., B.M., Writing: S.A., E.T.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Kennedy S, Bergqvist A, Chapron C, D'Hooghe T, Dunselman G, Greb R, et al. ESHRE guideline for the diagnosis and treatment of endometriosis. Hum Reprod. 2005;20:2698-2704. [Crossref]
2. Giudice LC, Kao LC. Endometriosis. Lancet. 2004;364:1789-1799. [Crossref]
3. Balasch J, Creus M, Fábregues F, Carmona F, Ordi J, Martinez-Román S, et al. Visible and non-visible endometriosis at laparoscopy in fertile and infertile women and in patients with chronic pelvic pain: a prospective syudy. Hum Reprod. 1996;11:387-391. [Crossref]
4. Exacoustos C, De Felice G, Pizzo A, Morosetti G, Lazzeri L, Centini G, et al. Isolated Ovarian Endometrioma: A History Between Myth and Reality. J Minim Invasive Gynecol. 2018;25:884-891. [Crossref]
5. Zhang X, Yao H, Huang X, Lu B, Xu H, Zhou C. Nerve fibres in ovarian endometriotic lesions in women with ovarian endometriosis. Hum Reprod. 2010;25:392-397. [Crossref]
6. Gerlinger C, Schumacher U, Wentzeck R, Uhl-Hochgräber K, Solomayer EF, Schmitz H, et al. How can we measure endometriosis-associated pelvic pain? J Endometriosis 2012;4:109-116. [Crossref]
7. Parasar P, Ozcan P, Terry KL. Endometriosis: Epidemiology, Diagnosis and Clinical Management. Curr Obstet Gynecol Rep. 2017;6:34-41. [Crossref]
8. Tsamantioti ES, Mahdy H. Endometriosis. [Updated 2022 Feb 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK567777/
9. Abdul Karim AK, Abd Aziz NH, Md Zin RR, Mohd Mokhtar N, Shafiee MN. The Effect of Surgical Intervention of Endometriosis to CA-125 and Pain. Malays J Med Sci. 2020;27:7-14. [Crossref]
10. Gelbaya TA, Nardo LG. Evidence-based management of endometrioma. Reprod Biomed Online. 2011;23:15-24.
11. Ballard KD, Seaman HE, de Vries CS, Wright JT. Can symptomatology help in the diagnosis of endometriosis? Findings from a national case-control study--Part 1. BJOG. 2008;115:1382-1391. [Crossref]
12. Donnez J, Nisolle M, Gillerot S, Smets M, Bassil S, Casanas-Roux F. Rectovaginal septum adenomyotic nodules: a series of 500 cases. Br J Obstet Gynaecol. 1997;104:1014-1018. [Crossref]
13. Wykes CB, Clark TJ, Chakravati S, Mann CH, Gupta JK. Efficacy of laparoscopic excision of visually diagnosed peritoneal endometriosis in the treatment of chronic pelvic pain. Eur J Obstet Gynecol Reprod Biol. 2006;125:129-133. [Crossref]
14. Practice Committee of the American Society for Reproductive Medicine. Treatment of pelvic pain associated with endometriosis: a committee opinion. Fertil Steril. 2014;101:927-935. [Crossref]

15. Tokushige N, Markham R, Russell P, Fraser IS. Nerve fibres in peritoneal endometriosis. Hum Reprod. 2006;21:3001-3007. [Crossref]
16. Wang G, Tokushige N, Markham R, Fraser IS. Rich innervation of deep infiltrating endometriosis. Hum Reprod. 2009;24:827-834.
17. Tokushige N, Russell P, Black K, Barrera H, Dubinovsky S, Markham R, et al. Nerve fibers in ovarian endometriomas. Fertil Steril. 2010;94:1944-1947. [Crossref]
18. Odagiri K, Konno R, Fujiwara H, Netsu S, Yang C, Suzuki M. Smooth muscle metaplasia and innervation in interstitium of endometriotic lesions related to pain. Fertil Steril. 2009;92:1525-1531. [Crossref]
19. Kang SB, Chung HH, Lee HP, Lee JY, Chang YS. Impact of diagnostic laparoscopy on the management of chronic pelvic pain. Surg Endosc. 2007;21:916-919. [Crossref]
20. Sasamoto N, DePari M, Vitonis AF, Laufer MR, Missmer SA, Shafrir AL, et al. Evaluation of CA125 in relation to pain symptoms among adolescents and young adult women with and without surgically-confirmed endometriosis. PLoS One. 2020;15:e0238043. [Crossref]
21. Hirsch M, Duffy JMN, Deguara CS, Davis CJ, Khan KS. Diagnostic accuracy of Cancer Antigen 125 (CA125) for endometriosis in symptomatic women: A multi-center study. European J Obstet Gynecol Reprod Biol. 2017;210:102-107. [Crossref]
22. Nisenblat V, Bossuyt PM, Shaikh R, Farquhar C, Jordan V, Scheffers CS, et al. Blood biomarkers for the non-invasive diagnosis of endometriosis. Cochrane Database Syst Rev. 2016;2016:CD012179. [Crossref]
23. Stratton P, Berkley KJ. Chronic pelvic pain and endometriosis: translational evidence of the relationship and implications. Hum Reprod Update. 2011;17:327-346. [Crossref]

ORIGINAL ARTICLE

DOI: 10.4274/hamidiyemedj.galenos.2022.18480

Hamidiye Med J 2022;3(2):153-158

Clinical Reflection of Pre- and Post-COVID-19 Symptoms in COVID-19 Patients

COVID-19 Hastalarında Hastalık Öncesi ve Sonrası Semptomların Klinik Yansıması

Barış Demirkol[1], Şule Gül[2], Mustafa Çörtük[2], Aysu Sinem Koç[3], Umut İlhan[2], Kürşad Nuri Baydili[4], Erdoğan Çetinkaya[2]

[1]*University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital, Clinic of Chest Diseases, İstanbul, Türkiye*

[2]*University of Health Sciences Türkiye, Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital, Clinic of Chest Diseases, İstanbul, Türkiye*

[3]*İstinye University, Bahçeşehir Liv Hospital, Clinic of Chest Diseases, İstanbul, Türkiye*

[4]*University of Health Sciences Türkiye Hamidiye Faculty of Medicine, Department of Biostatistics and Medical Informatics, İstanbul, Türkiye*

ABSTRACT

Background: Hospitalized or non-hospitalized patients with Coronavirus disease-2019 (COVID-19) may exhibit different symptoms. Different symptoms of patients can give us an idea about the course of the disease.

Materials and Methods: A cross-sectional questionnaire study was conducted between August and October 2021. The questionnaire was sent online to patients who had COVID-19 infection with polymerase chain reaction positivity. The following categories were included in the questionnaire: Demographic characteristics, diagnosis date, initial symptoms, predominant symptoms, treatments, presence of hospitalization requirement, ongoing symptoms after the treatment, and symptom duration.

Results: Of the 596 patients, 574 (92.4%) were symptomatic at the beginning of the disease. Myalgia, fatigue, and headache were the most prominent initial symptoms. Fever and dyspnea were the most predominant symptoms that compel the patients to apply to the hospital. While dyspnea was significantly higher in hospitalized patients, fever was more common in non-hospitalized patients. Higher mean age, male gender, and comorbidity (especially chronic obstructive pulmonary disease) were found to be factors that increased hospitalization ($p<0.01$). Fever and fatigue were mostly seen among females, while myalgia was most prominent among men. Four hundred and seventy-seven patients (80%) had post-COVID symptoms and the most common symptoms were getting tired easily, fatigue, myalgia, and cough. However, post-COVID symptoms were most intense in the second month and there were cases with complaints up to one year.

Conclusion: The majority of the COVID-19 patients in our study were symptomatic. Dyspnea and fever with higher mean age were more common in patients requiring hospitalization. Post-COVID symptoms may persist for a long time, and long-term monitoring may be beneficial.

Keywords: COVID-19, hospitalization, symptom

ÖZ

Amaç: Hastaneye yatışı olan ya da ayaktan tedavi alan Koronavirüs hastalığı-2019 (COVID-19) hastaları farklı semptomlar gösterebilir. Bu semptom farklılığı, hastalığın gidişatı hakkında bilgi öngörebilir.

Gereç ve Yöntemler: Ağustos-Ekim 2021 tarihleri arasında kesitsel anket çalışması uygulandı. Polimeraz zincir reaksiyonu pozitifliği ile COVID-19 hastalığı tanısı konan hastalara, anket formu online olarak gönderildi. Ankette yer alan sorular; demografik özellikler, hastalık tanı tarihi, ilk semptom, baskın semptom, alınan tedaviler, hastane yatışı, tedavi sonrası devam eden semptomlar ve semptom süresi ile ilgili bilgileri içermekteydi.

Bulgular: Hastalığın başlangıcında, 596 hastanın 574'ünün (%92,4) semptomu mevcuttu. En sık görülen başlangıç semptomları, kas ağrısı, halsizlik ve baş ağrısıydı. Ateş ve dispne, hastanın hastaneye başvurmasına neden olan en baskın semptom olarak saptandı.

Address for Correspondence: Barış Demirkol, University of Health Sciences Türkiye, Başakşehir Çam and Sakura City Hospital, Clinic of Chest Diseases, İstanbul, Türkiye
Phone: +90 541 733 48 07 E-mail: barisdemirkol34@gmail.com **ORCID ID:** orcid.org/0000-0001-5585-3842

Received: 24.03.2022 **Accepted:** 07.07.2022

©Copyright 2022 by University of Health Sciences Türkiye Hamidiye Faculty of Medicine. / Hamidiye Medical Journal published by Galenos Yayınevi.

ÖZ

Dispne hastane yatışı olan hastalarda daha sık iken, ateş ayaktan tedavi alan hastalarda daha sık saptandı. Ortalama yaşın yüksekliği, erkek cinsiyet ve komorbidite varlığı (özellikle kronik obstrüktif akciğer hastalığı) hastane yatışını artıran faktörler olarak gözlendi (p<0,01). Ateş ve halsizlik en sık kadın hastalarda görülürken, kas ağrısı erkeklerde daha sıktı. Dört yüz yetmiş yedi (%80) hastanın post-COVID semptomu mevcuttu ve en sık görülen semptomlar; çabuk yorulma, halsizlik, kas ağrısı ve öksürüktü. Post-COVID semptomlar en çok 2. ayda görülmekteydi fakat 1 yılı aşkın semptomu devam eden olgularımız da mevcuttu.

Sonuç: Çalışmamızda, COVID-19 hastalarının çoğunluğunun en az bir semptomu vardı. Hastane yatışı gereken hastalarda, ortalama yaş daha yüksek ve ateş ve dispne en sık görülen semptomlardı. Post-COVID dönemde semptomların uzun süre devam edebileceği gözlendi, bu nedenle hastaların uzun dönem takibi faydalı olabilir.

Anahtar Kelimeler: COVID-19, hastane yatışı, semptom

Introduction

Since the first case was detected in Wuhan, China in 2019, the Coronavirus disease-2019 (COVID-19) has affected 243 million people around the World up to February 2021 (1). Severe acute respiratory syndrome (SARS)-coronavirus (CoV)-2, which is the cause of COVID-19, affects the upper and lower respiratory tract and causes a high viral load in the upper respiratory tract. The rapid human-to-human spread of SARS-CoV-2 causes a wide spectrum of clinical manifestations in patients with COVID-19 (2).

In a study involving 11 centers in the United States of America, the most common symptoms related to COVID-19 infection were fever, body aches, headache, dyspnea, weakness, nausea, diarrhea, respectively. It has been shown that symptoms such as loss of taste and smell, sore throat, and headache are more common in outpatients, while dyspnea symptoms are more common in hospitalized patients. Fever, muscle pain, cough was observed at similar rates in both groups (3). Symptoms can persist in the post-COVID period in many patients. In outpatients, symptoms persisted in 27.8% at 4 months and 34.8% at 7 months of the post-COVID period (4). In hospitalized patients, the symptoms can continue in 94.9% of the patients in the post-COVID period (5).

In our study, the aim was to represent the initial and predominant symptoms of COVID-19 patients diagnosed by polymerase chain reaction (PCR) test and to investigate the relation of symptoms on hospitalization and ongoing symptom development in the post-COVID period.

Material and Methods

This cross-sectional questionnaire study was conducted at a tertiary chest diseases hospital between August and October 2021. A questionnaire was sent online to patients who had COVID-19 before the survey date. Inclusion criteria were: (1) Being aged 18 or higher, (2) COVID-19 infection diagnosis by PCR test, (3) agreeing to participate in the study. A voluntary consent form was added to the questionnaire and those who were approved were included in the study. Eligible patients were asked to fill out a questionnaire consisting of 20 questions. The questionnaire included the following: Demographic characteristics, diagnosis date, initial symptoms at diagnosis, predominant symptoms before hospital admission, treatments, presence of hospitalization requirement, ongoing symptoms after the treatment, and symptom duration.

Ethical approval for this study was obtained from the University of Health Sciences Türkiye Hamidiye Faculty of Medicine Ethics Committee with the decision number 5/15 on 05.02. 2021.

Statistical Analysis

The analysis of the data was carried out with the SPSS 25 package program. Frequency and percentage values for qualitative variables, arithmetic mean and standard deviation values for quantitative variables are presented. The chi-square test and Fisher's Exact test were used for comparisons between the two qualitative variables. An independent sample t-test was used for comparisons between qualitative variable categories in terms of quantitative variables. In determining the factors affecting hospitalization, variables that were found significantly as a result of pairwise comparisons were evaluated by binary logistic regression analysis. The type I error rate was taken as 0.05 in the study.

Results

A total of 596 patients questionnaire were included in the study. Demographic data of the patients and their effect on hospitalization are summarized in Table 1. The hospitalization rate was 16.1% (n=96). Thirteen (2.2%) patients were treated at the intensive care unit (ICU) and eight (1.3%) patients had been intubated. Advanced age, male gender, and comorbidity were found to be factors that increased hospitalization (p<0.01). In comorbidities, patients with chronic obstructive pulmonary disease (COPD) were

hospitalized more, while patients with diabetes received more outpatient treatment (Table 1).

In the questionnaire, patients were asked about the initial symptoms they experienced due to COVID-19 infection: High fever in 70 patients (25.3%), loss of taste and smell in 47 patients (16.2%), cough in 45 patients (15.4%), fatigue in 102 patients (33.6%), myalgia in 132 patients (42.8%) and headache in 88 patients (28.8%) were detected. These are the predominant initial symptoms. Twenty-two patients (7.6%) had no symptoms at the beginning.

Another question was about the predominant symptom that compels the patient to apply to the hospital. The most common predominant symptoms, the effect of symptom status on hospitalization, and differences between genders are given in Table 2. Fever and dyspnea are the most common predominant symptoms. While dyspnea was significantly higher in hospitalized patients, fever was more common in non-hospitalized patients. Fever and fatigue were mostly seen among females, while myalgia was the most prominent among men. When the predominant symptom status and age were considered together, it was found that the hospitalization rate was statistically significantly higher in those with advanced age and fever and those with advanced age and dyspnea (p<0.001) (Table 3).

Logistic regression analysis was performed to determine the factors affecting hospitalization status. As a result of the analysis, while the result of the Hosmer and Lemeshow test was 0.630, all p-values of the model were found to be <0.001. Being male was found to be a 1.961-fold risk factor (p=0.006), having a complaint of predominant dyspnea was a 9.752-fold risk factor (p=0.012), and a 1-unit increase in age was found to be a 1.052-fold risk factor (p<0.001) (Table 4).

Four hundred and seventy-seven patients (80%) had post-COVID symptoms. Symptom duration can be seen in Figure 1. Most patients (n=176-38.5%) had symptoms between the first and second months after COVID-19 infection. The most common post-COVID symptoms and duration of symptoms were summarized in Table 5. Getting tired easily, fatigue, myalgia, and cough were the most common post-COVID symptoms. The longest-lasting symptoms were: Dyspnea, fatigue, myalgia, and getting tired easily, respectively. It was found that dyspnea and getting tired easily were found to be statistically more common in hospitalized patients compared to those without hospitalization (p<0.001).

Discussion

In our study, advanced age, male gender, and comorbidities were found to be risk factors for hospitalization of COVID-19 patients. In comorbidities, COPD was a statistically significant risk factor for hospitalization. Dyspnea was the

Table 1. Demographic characteristics of COVID-19 patients

	Total n (%)	Hospitalization No n (%)	Hospitalization Yes n (%)	p
Age (mean ± SD)	38.93±12.07	37.48±11.46	46.47±12.40	**<0.001**
Gender				
Male	234 (39.3)	182 (77.8)	52 (22.2)	**<0.001**
Female	362 (60.7)	318 (87.8)	44 (12.2)	
Comorbidities				
No	398 (66.8)	348 (87.4)	50 (12.6)	**0.001**
Yes	198 (33.2)	152 (76.8)	46 (23.2)	
Asthma	38 (6.4)	33 (86.8)	5 (13.2)	0.777
COPD	7 (1.2)	3 (42.9)	4 (57.1)	**0.014**
Coronary artery disease	13 (2.2)	10 (76.9)	3 (23.1)	0.757
Diabetes	33 (5.5)	22 (66.7)	11 (33.3)	**0.012**
Hypertension	56 (9.4)	44 (78.6)	12 (21.4)	0.344
Chronic kidney disease	2 (0.3)	1 (50)	1 (50)	0.732
Congestive heart failure	3 (0.5)	2 (66.7)	1 (33.3)	0.979
Malignancy	2 (0.3)	1 (50)	1 (50)	0.732
Rheumatologic disease	39 (6.5)	31 (79.5)	8 (20.5)	0.583
Other	70 (11.7)	52 (74.3)	18 (25.7)	**0.031**

COPD: Chronic obstructive pulmonary disease, SD: Standard deviation, COVID-19: Coronavirus disease-2019

major predominant symptom and statistically significant symptom for hospitalization. Also, the hospitalization rate was higher in those with advanced age and predominant fever. The shortness of breath, fatigue, muscle pain, and cough were the most common post-COVID symptoms and the post-COVID symptoms were mostly seen in the second month.

Over 90% of patients with COVID-19 infection are symptomatic and have at least one symptom in several studies (3,6,7). Similar to the literature, 92.4% of the cases

Table 2. Effect of predominant symptoms to hospitalization and differences between genders

Dominant symptom		Hospitalization No n (%)	Hospitalization Yes n (%)	p	Male n (%)	Female n (%)	p
Fever	No	380 (85.8)	63 (14.2)	0.045	192 (82.1)	251 (69.3)	**0.001**
	Yes	120 (78.4)	33 (21.6)		42 (17.9)	111 (30.7)	
Loss of smell, taste or both	No	498 (83.8)	96 (16.2)	1.000	232 (99.1)	362 (100)	0.300
	Yes	2 (100)	0 (0)		2 (0.9)	0 (0)	
Cough	No	500 (84)	95 (16)	0.356	233 (99.6)	362 (100)	0.826
	Yes	0 (0)	1 (100)		1 (0.4)	0 (0)	
Fatigue	No	452 (83.4)	90 (16.6)	0.394	221 (94.4)	321 (88.7)	**0.024**
	Yes	48 (88.9)	6 (11.1)		13 (5.6)	41 (11.3)	
Myalgia	No	490 (83.6)	96 (16.4)	0.325	226 (96.6)	360 (99.4)	**0.020**
	Yes	10 (100)	0 (0)		8 (3.4)	2 (0.6)	
Skin rash	No	495 (83.9)	95 (16.1)	1.000	231 (98.7)	359 (99.2)	0.903
	Yes	5 (83.3)	1 (16.7)		3 (1.3)	3 (0.8)	
Dyspnea	No	498 (84.7)	90 (15.3)	**<0.001**	231 (98.7)	357 (98.6)	1.000
	Yes	2 (25)	6 (75)		3 (1.3)	5 (1.4)	

Table 3. Effect of predominant symptom and age together to hospitalization

Dominant symptom	Hospitalization No $\bar{x}$ ± SD	Hospitalization Yes $\bar{x}$ ± SD	t	p
Fever	36.52±11.14	46.11±11.91	-8.928	**<0.001**
Fatigue	38.92±12.18	39.09±10.95	-0.101	0.919
Myalgia	38.98±12.09	36.4±11.1	0.669	0.504
Skin rash	38.94±12.1	38.33±9	0.122	0.903
Dyspnea	38.7±11.9	56.13±12.5	-4.111	**<0.001**

SD: Standard deviation

Table 4. Results of logistic regression analysis to determine the factors affecting hospitalization status

	B	S.E.	Wald	p	OR (95% CI)
Gender (ref: Female)	-0.673	0.247	7.459	**0.006**	1.961 (0.209-3.179)
COPD	0.739	0.907	0.664	0.415	2.094 (0.354-12.39)
Diabetes	0.497	0.476	1.089	0.297	1.643 (0.646-4.176)
Other comorbidities	0.606	0.324	3.5	0.061	1.833 (0.972-3.457)
Fever	0.007	0.319	0.001	0.982	1.007 (0.539-1.882)
Dyspnea	2.277	0.909	6.278	**0.012**	9.752 (1.642-57.911)
Age	0.051	0.012	19.491	**<0.001**	1.052 (1.029-1.076)
Constant	-1.865	0.954	3.821	0.051	0.155

COPD: Chronic obstructive pulmonary disease, OR: Odds ratio, CI: Confidence interval

had an initial symptom in our study. Previous studies reported that, the most common symptoms were: Fever, myalgia, weakness, dyspnea, loss of taste and smell, and headache, respectively. Although gastrointestinal symptoms such as diarrhea were observed at rates ranging from 5% to 30% in studies, diarrhea and GIS symptoms were not observed in our study (3,8,9).

Fever and fatigue were mostly seen in females, myalgia was mostly seen in men. There are different results in the studies about gender and symptoms. In the study of Tandan et al. (10), while malaise/body soreness, cough, anorexia, and headache were more common in men, sorethroat and rhinorrhea was more common in women. Fever was seen equally in both genders. In another study, while loss of smell, headache, nasal obstruction, throat pain, and fatigue were mostly seen in females, males more from cough and fever. Differences between the genders have been attributed to factors related to different immune systems, steroid hormones, and sex hormones. Immune regulatory genes on the X-chromosome have been shown to cause lower viral load and less inflammation than men (11).

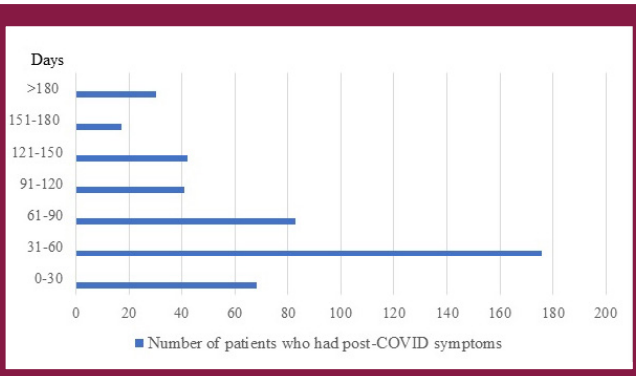

Figure 1. Number of patients who had post-COVID symptoms according to post-COVID days
COVID-19: Coronavirus disease-2019

Table 5. Post-COVID symptoms

Post-COVID symptoms	n (%)	Symptom duration-day (Median/min-max)
Fever	1 (0.2)	-
Loss of weight	70 (11.7)	51 (4-239)
Anorexia	14 (2.3)	58 (31-153)
Diarrhea	4 (0.7)	-
Cough	71 (11.9)	43 (4-135)
Fatigue	152 (25.5)	55 (9-354)
Myalgia	114 (19.1)	66 (10-354)
Headache	56 (9.4)	67 (9-274)
Skin rash	11 (1.8)	43 (24-267)
Dyspnea	65 (10.9)	71 (19-354)
Getting tired easily	197 (33.1)	55 (9-290)
Other	56 (9.4)	51 (11-273)
No symptom	119 (20)	59 (6-358)

COVID: Coronavirus

In studies examining the relationship between symptoms and hospitalization, it was found that dyspnea was more common in patients who required hospitalization. Fever was generally found at similar rates in the two groups (3,6,7). In our study, predominant dyspnea was the most common symptom in hospitalized patients, while predominant fever was more common in non-hospitalized patients. However, when associated with age, it was found that hospitalization was more common in those with both dyspnea and fever symptoms in advanced age.

As summarized in the review of Gallo Marin et al. (12), advanced age, male gender, and comorbidities such as diabetes, hypertension, respiratory diseases (especially COPD) are related to hospital and intensive care stays in COVID-19 disease, and the disease progresses more seriously in these patients. In our study, hospitalization was higher in patients with advanced age and male gender. Considering the comorbidities, COPD was associated with a statistically significant level of hospitalization. Unlike the data in the literature, diabetes was observed more frequently in patients without hospitalization. This may be due to the fewer number of hospitalized patients in our study.

Iqbal et al. (5) showed that 94.9% of patients experienced at least one post-COVID symptom after COVID infection and the most commonly observed symptom was fatigue. In a different study, the most common post-COVID symptoms in non-hospitalized patients were: Shortness of breath, anosmia, ageusia, and fatigue, respectively (4). In our study, 80% of patients had post-COVID symptoms and the most common post-COVID symptoms were getting tired easily and fatigue, similar to the literature. In a study in which SARS cases were followed for around 4 years, it was observed that 40.3% of patients had chronic fatigue complaints (13). The other most common post-COVID symptom seen in these studies was dyspnea/shortness of breath, which was still observed in the sixth month of studies (5,14). In our study, although the duration of symptoms was most intense in the second month of post-COVID, there were cases with complaints of up to one year.

In a study conducted in China, post-COVID-19 patients who received and did not receive pulmonary rehabilitation were evaluated and after 6 months of pulmonary rehabilitation, a significant increase was found in the 6-minute walking test in the group receiving pulmonary rehabilitation (15). Close follow-up and, if necessary, rehabilitation may be helpful

against fatigue and dyspnea symptoms after COVID-19 infection.

Study Limitations

Our study has some limitations. Firstly, it was a single-center study. The population of the study was not randomized and consisted mostly of non-hospitalized patients. Since our study was a questionary study, the results were based on the answers given by the patients, and data such as radiological or pulmonary function tests could not be used to confirm these results. We also do not have the data for COVID-19 variants.

Conclusion

Dyspnea is the most common symptom with hospitalized COVID-19 patients. Advanced age, male gender, and COPD in comorbidities were found to be risk factors for hospitalization. In the post-COVID period, fatigue, and dyspnea can be seen in many patients and can last for a long time.

Information: The article was presented as an oral presentation at the National Lung Health Congress 16-19 March 2022, Belek, Antalya, SS-115.

Ethics

Ethics Committee Approval: Ethical approval for this study was obtained from the University of Health Sciences Türkiye Hamidiye Faculty of Medicine Ethics Committee with the decision number 5/15 on 05.02. 2021.

Informed Consent: A voluntary consent form was added to the questionnaire and those who were approved were included in the study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.D., Ş.G., M.Ç., A.S.K., U.İ., E.Ç., Concept: B.D., Ş.G., M.Ç., U.İ., K.N.B., E.Ç., Design: B.D., Ş.G., M.Ç., A.S.K., U.İ., K.N.B., E.Ç., Data Collection or Processing: B.D., M.Ç., U.İ., K.N.B., Analysis or Interpretation: B.D., Ş.G., M.Ç., A.S.K., K.N.B., E.Ç., Literature Search: B.D., Ş.G., A.S.K., K.N.B., Writing: B.D., Ş.G., M.Ç., E.Ç.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. World Health Organization. COVID-19 weekly epidemiological update. World Heal Organ [Internet]. 2021;1-23. Available from: https://www.who.int/publications/m/item/covid-19-weekly-epidemiological-update
2. Raoult D, Zumla A, Locatelli F, Ippolito G, Kroemer G. Coronavirus infections: Epidemiological, clinical and immunological features and hypotheses. Cell Stress. 2020;4:66-75. [Crossref]
3. Tenforde MW, Billig Rose E, Lindsell CJ, Shapiro NI, Files DC, Gibbs KW, et al. Characteristics of Adult Outpatients and Inpatients with COVID-19 - 11 Academic Medical Centers, United States, March-May 2020. MMWR Morb Mortal Wkly Rep. 2020;69:841-846. [Crossref]
4. Augustin M, Schommers P, Stecher M, Dewald F, Gieselmann L, Gruell H, et al. Post-COVID syndrome in non-hospitalised patients with COVID-19: a longitudinal prospective cohort study. Lancet Reg Health Eur. 2021;6:100122. [Crossref]
5. Iqbal A, Iqbal K, Arshad Ali S, Azim D, Farid E, Baig MD, et al. The COVID-19 Sequelae: A Cross-Sectional Evaluation of Post-recovery Symptoms and the Need for Rehabilitation of COVID-19 Survivors. Cureus. 2021;13:e13080. [Crossref]
6. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395:497-506. [Crossref]
7. Mei X, Zhang Y, Zhu H, Ling Y, Zou Y, Zhang Z, et al. Observations about symptomatic and asymptomatic infections of 494 patients with COVID-19 in Shanghai, China. Am J Infect Control. 2020;48:1045-1050. [Crossref]
8. Liu K, Fang YY, Deng Y, Liu W, Wang MF, Ma JP, et al. Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei Province. Chin Med J (Engl). 2020;133:1025-1031. [Crossref]
9. Gattinoni L, Gattarello S, Steinberg I, Busana M, Palermo P, Lazzari S, et al. COVID-19 pneumonia: pathophysiology and management. Eur Respir Rev. 2021;30:210138. [Crossref]
10. Tandan M, Acharya Y, Pokharel S, Timilsina M. Discovering symptom patterns of COVID-19 patients using association rule mining. Comput Biol Med. 2021;131:104249. [Crossref]
11. Lechien JR, Chiesa-Estomba CM, Place S, Van Laethem Y, Cabaraux P, Mat Q, et al. Clinical and epidemiological characteristics of 1420 European patients with mild-to-moderate coronavirus disease 2019. J Intern Med. 2020;288:335-344. [Crossref]
12. Gallo Marin B, Aghagoli G, Lavine K, Yang L, Siff EJ, Chiang SS, et al. Predictors of COVID-19 severity: A literature review. Rev Med Virol. 2021;31:1-10. [Crossref]
13. Lam MH, Wing YK, Yu MW, Leung CM, Ma RC, Kong AP, et al. Mental Morbidities and Chronic Fatigue in Severe Acute Respiratory Syndrome Survivors: Long-term Follow-up. Arch Intern Med. 2009;169:2142-2147. [Crossref]
14. Fernández-De-las-peñas C, Palacios-Ceña D, Gómez-Mayordomo V, Cuadrado ML, Florencio LL. Defining post-covid symptoms (Post-acute covid, long covid, persistent post-covid): An integrative classification. Int J Environ Res Public Health. 2021;18:2621. [Crossref]
15. Dun Y, Liu C, Ripley-Gonzalez JW, Liu P, Zhou N, Gong X, et al. Six-month outcomes and effect of pulmonary rehabilitation among patients hospitalized with COVID-19: a retrospective cohort study. Ann Med. 2021;53:2099-2109. [Crossref]

CASE REPORT

DOI: 10.4274/hamidiyemedj.galenos.2022.29491

Hamidiye Med J 2022;3(2):159-161

A Case of Mycosis Fungoides Developed Early After Hodgkin Lymphoma Treatment

Hodgkin Lenfoma Tedavisi Sonrası Erken Dönemde Mikozis Fungoides Gelişmiş Olgu

Emrah Kılıçaslan, Muhammet Kürşat Kaptan

University of Health Sciences Türkiye, Sultan 2. Abdulhamid Han Training and Research Hospital, Clinic of Internal Medicine, Division of Hematology, İstanbul, Türkiye

ABSTRACT

Coexistence or sequentially of Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) can occur in the same patient rarely. Although the underlying etiopathogenesis of this condition is not known for certain, reasons such as drugs, genetic and environmental factors, and the effect of the primary tumor are blamed. Also, synchronous or metachronous formation of HL and T-cell NHL in the same patient is known, and this occurs most often with mycosis fungoides (MF). MF usually precedes HL, but to a much lesser extent, the opposite is observed. There are often years between the development of two separate diseases. A 38-year-old male presented swelling on the neck, high fevers, sweating, and weight loss. A patient diagnosed with stage 4B HL-nodular sclerosis type after evaluation. ABVD regimen was initiated for the patient and 6 cycles of chemotherapy were completed. Complete response was observed after six cycles of chemotherapy. He did well for the next 2 months but then developed cutaneous lesions like slightly hypopigmented and dandruff patches on the trunk and MF diagnosed after skin biopsy. In HL patients, secondary malignancies such as MF may occur very soon after the end of treatment.

Keywords: Mycosis fungoides, Hodgkin lymphoma, secondary malignancy

ÖZ

Nadiren Hogdkin lenfoma (HL) ve non-Hodgkin lenfoma (NHL) birlikte veya sıralı olarak aynı hastada ortaya çıkabilmektedir. Bu durumun etiyopatogenezi kesin olarak bilinmemekle birlikte, ilaçlar, genetik ve çevresel faktörler, primer tümörün etkisi gibi nedenlere bağlı olabileceği düşünülmektedir. Aynı hastada HL ve T-hücreli NHL'ler de senkron veya metakron gelişebilmektedir. Bu durum T-hücreli NHL'ler içinde en sık mikozis fungoides (MF) ile ortaya çıkmaktadır. Her iki hastalığı da barındıran kişilerde MF genellikle HL'den daha önce gelişmektedir, ancak çok daha az oranda bu durumun tersi gözlenebilmektedir. Literatürde bildirilen olgularda iki ayrı hastalığın gelişim süreleri arasında genellikle yıllar vardır. Otuz sekiz yaşında erkek hasta boyunda şişlik, yüksek ateş, terleme ve kilo kaybı ile başvurdu. Tanısal değerlendirmelerden sonra evre 4B HL-nodüler sklerozan tip tanısı konuldu. ABVD rejimi ile kemoterapiye başlandı ve 6 siklus kemoterapiyi tamamladı. Tedavi sonrası yapılan incelemede tam yanıtlı olarak değerlendirildi. Tedavi bittikten 2 ay sonra hastanın derisinde hafif hipopigmente lezyonlar çıktı. Deri lezyonlarının patolojik incelemesi sonrası MF tanısı konuldu. HL hastalarında tedavi bitiminden çok kısa bir süre sonra MF gibi sekonder maligniteler ortaya çıkabilmektedir.

Anahtar Kelimeler: Mikozis fungoides, Hodgkin lenfoma, ikincil malignite

Address for Correspondence: Emrah Kılıçaslan, University of Health Sciences Türkiye, Sultan 2. Abdulhamid Han Training and Research Hospital, Clinic of Internal Medicine, Division of Hematology, İstanbul, Türkiye

Phone: +90 555 678 66 54 E-mail: dremrahkilicaslan@gmail.com **ORCID ID:** orcid.org/0000-0002-0944-4068

Received: 21.04.2022 **Accepted:** 03.06.2022

©Copyright 2022 by University of Health Sciences Türkiye Hamidiye Faculty of Medicine. / Hamidiye Medical Journal published by Galenos Yayınevi.

Introduction

Hodgkin lymphoma (HL), anciently known as Hodgkin's disease, is a hematological malignant disease that develops from germinal or post-germinal center B lymphocytes. The disease has a cellular combination, including neoplastic cells (Reed-Sternberg cells and their variants) in the inflamed environment. HL is less common than non-Hodgkin lymphoma (NHL) and constitutes 10% of lymphomas in all over the world. It also makes up 0,6% of total cancer cases in developed countries (1).

Mycosis fungoides (MF) is a T-cell non-Hodgkin lymphoma (T-NHL) presenting in the derm but with possible involvement of the blood, lymph nodes, and internal organs. Skin lesions observed in the disease include patches or plaques, which may be localized or diffuse. The etiopathogenesis of MF is not known exactly, although genetic and epigenetic factors have been implicated. Although environmental and occupational exposure to solvents and chemicals played a role in the etiology of the disease, a large case-controlled study did not support this hypothesis (2).

The association between MF and HL was reported for the first time in 1963 (3). In patients who harbor both malignancies; MF usually precedes HL, but is much less likely vice versa (4). In addition, we see that MF develops years after HL in cases reported in the literature.

Case Report

A thirty-eight years old male patient presented with enlargement in the neck region and B symptoms (fever, night perspiration, and 8 kg involuntary weight loss in the last six months). No disease or suspicious finding was found in the patient's medical history. Physical examination revealed left cervical and supraclavicular lymphadenopathy. No abnormal finding was found in other systems in physical examination. In laboratory evaluation, sedimentation rate was 13 mm/h, lactate dehydrogenase: 297 U/L, while other biochemical parameters were within normal limits.

Excisional biopsy was taken from the left cervical lymph node, and as a result of a pathological examination, it was reported as classical HL nodular sclerosis type (CD30+, EBV+, FASCIN+).

Positron emission tomography/computed tomography (PET/CT) scanning showed involved lymph nodes in the left submandibular, left infraclavicular, mediastinal, and abdominal areas. In addition, multiple disease involvement areas were observed in the liver and pelvic bones. No disease involvement was observed in the bone marrow biopsy examination. As a result of all diagnostic examinations, the patient was diagnosed with stage 4B HL. The patient was started on doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) chemotherapy regimen. Six cycles of scheduled chemotherapy were administered to the patient without complications. It was observed that there was a complete response to the treatment in the PET/CT scanning taken after the treatment.

In the following period, he did not have any complaints for 2 months. However, later on, multiple mildly hypopigmented cutaneous lesions such as dandruff spots developed on the trunk of the patient (Figure 1). The patient was then referred to the dermatology department of the institute. A diagnosis of MF was made as a result of a skin biopsy of the lesion.

Discussion

HL cases treated with chemotherapy and/or radiation therapy are more likely to develop solid tumors or hematological malignancies later in life than the general population (5). Due to the new and effective drugs discovered in cancer treatment, it is estimated that the life expectancy of these patients will be prolonged, and secondary malignancies will be observed more frequently in the future.

It has been reported many times that HL and NHL coexisted at identical or different times in the same

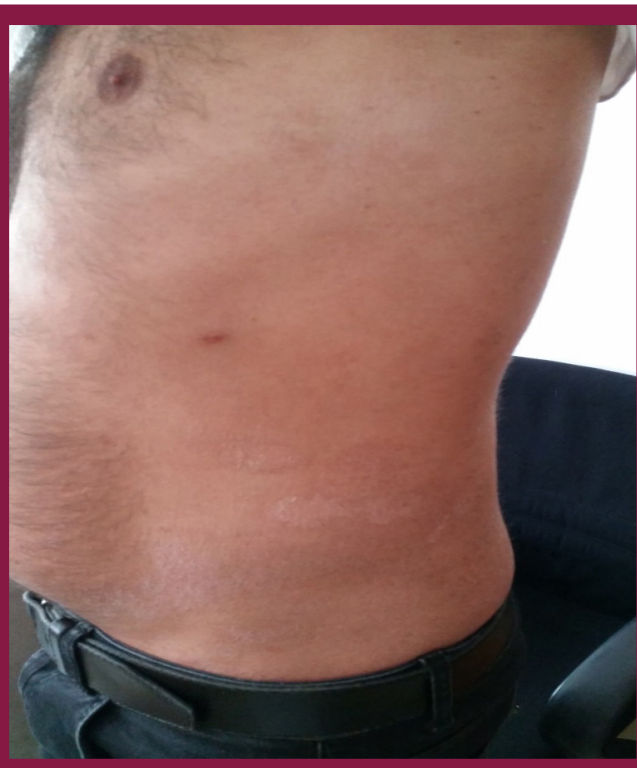

Figure 1. Mycosis fungoides lesions on the patient's skin

patient. Two neoplasms can emerge concurrently. Also, the emergence of HL may be before or after the onset of NHL.

Simultaneous development of HL and T-NHL in one patient has also been reported previously. The best frequently identified association is between HL and MF (6). In reported cases, MF is generally diagnosed prior to HL, but the opposite situation has also been reported rarely. Lipa et al. (4) reported that MF developed in two patients diagnosed with HL years after the administration of chemotherapy for HL.

Amongst peripheral T-NHL, the most common malignancy related to HL is MF. However, most of these patients are cases that develop HL after being followed and treated for a long time with the diagnosis of MF. This has raised the suspicion of some scientists that MF and HL arise from the same progenitor cell (7). However, another study on immunological antigen expression patterns and gene rearrangements in HL and cutaneous T-cell lymphoma suggests that malignancies do not originate from the same cell (6).

There are different opinions in the scientific community about the emergence of secondary malignant neoplasms. Some scientists argue that viral pathogens, potential mutagenic effects of chemotherapeutic drugs administered to the patient, or genetic tendency in the patient may contribute to the development of B and T-cell malignancies in the same individual. Barzilai et al. (8) suggested that cytokines released by existing malignant cells may lead to a secondary malignancy with a carcinogenic effect on progenitor stem cells. In a previous study, Väkevä et al. (9) found a high risk of HL and NHL as secondary cancers in the follow-up of 319 patients with cutaneous T-cell lymphoma. However, they could not comment on the pathogenesis of secondary malignancy development.

Combined modality therapy for HL significantly increases the risk of developing secondary malignancies compared to chemotherapy alone. It is known that people exposed to alkylating agents and/or topoisomerase II inhibitors are at increased risk of growing acute myeloid leukemia or myelodysplastic syndrome later in life (10). However, such relationships have not been defined for secondary NHL. Both drug classes form the backbone of chemotherapy protocols such as ABVD, BEACOPP, and stanford V applied in the treatment of HL.

The development of secondary hematological neoplasia usually occurs within the first 10 years after the diagnosis of HL. However, the risk of secondary solid malignancy increases significantly after 25 years. MF lesions developed in our patient 2 months after receiving the last course of chemotherapy for the treatment of HL. Did our patient have MF skin lesions before the diagnosis of HL? This question occupied our minds a lot. However, our patient was sure that the skin lesions were new. When we conducted a literature search, we did not encounter MF that developed so early in a patient with HL.

In conclusion, the physician should bear in mind that secondary hematological malignancies such as MF may occur not only in the late-term but also in the very early term in treated HL patients.

Ethics

Informed Consent: Informed consent was obtained.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.K., M.K.K., Concept: E.K., M.K.K., Design: E.K., M.K.K., Data Collection or Processing: E.K., M.K.K., Analysis or Interpretation: E.K., M.K.K., Literature Search: E.K., M.K.K., Writing: E.K., M.K.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70:7-30. [Crossref]
2. Whittemore AS, Holly EA, Lee IM, Abel EA, Adams RM, Nickoloff BJ, et al. Mycosis fungoides in relation to environmental exposures and immune response: a case-control study. J Natl Cancer Inst. 1989;81:1560-1567. [Crossref]
3. Block JB, Edgcomb J, Eisen A, Van Scott EJ. Mycosis fungoides. Natural history and aspects of its relationship to other malignant lymphomas. Am J Med. 1963;34:228-235. [Crossref]
4. Lipa M, Kunynetz R, Pawlowski D, Kerbel G, Haberman H. The occurrence of mycosis fungoides in two patients with preexisting Hodgkin's disease. Arch Dermatol. 1982;118:563-567. [Crossref]
5. Canellos GP, Arseneau JC, DeVita VT, Whang-Peng J, Johnson RE. Second malignancies complicating Hodgkin's disease in remission. Lancet. 1975;1:947-949. [Crossref]
6. Brousset P, Lamant L, Viraben R, Schlaifer D, Gorguet B, Duhault P, et al. Hodgkin's disease following mycosis fungoides: phenotypic and molecular evidence for different tumour cell clones. J Clin Pathol. 1996;49:504-507. [Crossref]
7. Davis TH, Morton CC, Miller-Cassman R, Balk SP, Kadin ME. Hodgkin's disease, lymphomatoid papulosis, and cutaneous T-cell lymphoma derived from a common T-cell clone. N Engl J Med. 1992;326:1115-1122. [Crossref]
8. Barzilai A, Trau H, David M, Feinmesser M, Bergman R, Shpiro D, et al. Mycosis fungoides associated with B-cell malignancies. Br J Dermatol. 2006;155:379-386. [Crossref]
9. Väkevä L, Pukkala E, Ranki A. Increased risk of secondary cancers in patients with primary cutaneous T cell lymphoma. J Invest Dermatol. 2000;115:62-65. [Crossref]
10. Eichenauer DA, Thielen I, Haverkamp H, Franklin J, Behringer K, Halbsguth T, et al. Therapy-related acute myeloid leukemia and myelodysplastic syndromes in patients with Hodgkin lymphoma: a report from the German Hodgkin Study Group. Blood. 2014;123:1658-1664. [Crossref]